

A brief inventory of bruchids (Chrysomelidae, Bruchinae) and similar pest beetles occurring on peas in the Republic of Congo: barcoding and prospects

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Abstract

The seed beetles of the family Chrysomelidae, subfamily Bruchinae, are pest species that cause substantial damage to legume crops, affecting supply for human and animal consumption. Legumes are a staple of the Congolese population due to their protein-rich seeds, productivity, and ability to improve soil fertility when combined with cereals. In this study, we focused on bruchids that attack two varieties of peas, *Cajanus cajan* and *Vigna unguiculata* subsp. *cylindrica*, in the Republic of Congo. 80 individuals of bruchids were considered from the two main pea-growing departments: Bouenza and Niari. Specimens were collected using baited traps placed in the field and by manually selecting affected seeds from the storage areas. Individuals were categorised at the species level based on morphological traits and molecular barcode analyses. This is the second time that 12S rRNA sequences of bruchids from the Republic of Congo have been deposited in the GenBank database. This study emphasises the need to enrich the GenBank database with DNA sequences originating from areas where the technical means to carry out quality morphological analyses are limited.

Introduction

Domestic Fabaceae cultivars, such as groundnuts, beans, and peas, are the most vulnerable to infestation by bruchids. In the Republic of Congo (RC), the production of these legumes is primarily a traditional concern, wherein most producers are smallholders grouped in agricultural cooperatives or family communities and maintain relatively low productivity.

Some of the most important factors limiting the productivity of Congolese agriculture are 1) the absence of crop protection systems and 2) the high level of post-harvest losses. These two factors result in consistently low yields, forcing the local markets to import legumes from Cameroon and consequently increase their prices. Post-harvest losses (fruit weight loss, reduced nutritional value, and impeded germination capacity) are mainly caused by small Coleoptera belonging to the Chrysomelidae family (Borowiec 1987; Delobel 2000; Keneni et al. 2011) . The most economically damaging genera are *Acanthoscelides*, *Bruchidius*, *Bruchus*, *Callosobruchus*, *Caryedon*, and *Zabrotes* (Johnson 1981, Huignard et al. 2011) . The affected seeds become unfit for human (1-3% threshold) and animal (10%) consumption and are unviable for planting due to reduced germination capacity. Although legume growers have difficulty estimating the level of crop loss to bruchids, it is generally claimed that insects cause the most damage to reserve stocks (Boxall 1986) .

A necessary prerequisite to reduce post-harvest losses and improve biosecurity is establishing an accurate database of Chrysomelidae pests that affect economically important legumes (FAOSTAT, 2010). For decades, morphospecies have been identified exclusively using morphological diagnostics provided by taxonomic studies (Borowiec 1987) .

This identification method requires specialised skills that are often acquired through long-term experience that only experts, such as taxonomists and qualified technicians, possess (Raupach et al. 2010). In view

of the decreasing number of experts (Agnarsson and Kuntner 2007) and the loss of biodiversity, an alternative method is needed to rapidly collate biodiversity inventories and allow species identification by non-specialists. Hebert et al. (2003) proposed a morphospecies identification method using a short mitochondrial DNA sequence. This tool makes it possible to identify insect species in their developmental stages (Bakhoun et al. 2018), morphospecies without sufficient discriminating characteristics, and cryptic species. The scientific value of barcoding has been shown in the biomonitoring of several insect pests that cause significant economic damage to important crops worldwide (Boykin et al. 2012; Ashfaq and Hebert 2016). Some authors have tested the effectiveness of mitochondrial cytochrome c oxidase gene I (COI) on nearly 7,237 sequences, representing 542 species of Chrysomelidae (Magoga et al. 2018). The barcoding efficiency was 94%, and 6% of the sequences were misidentified. Other molecular markers have been successfully developed since the article of Hebert et al. (2003) was published (Hebert et al. 2003), including 12S rRNA, Cytb, and ITSS (Tixier et al. 2006; Okassa et al. 2009, 2010; M Hernández-Triana et al. 2019).

From in 2021 (Dibangou et al. 2021), scientific studies began using barcoding to assess the biodiversity and distribution of insects belonging to the Chrysomelidae family in Congo. In fact, until recently, the only data available came from reports carried out by the Ministry of Scientific Research and the French Institute of Scientific Research for Development in Cooperation (Delobel and Epouna-Mouinga 1984) with a pre-survey was conducted in the Bouenza region on Farmers' storage structures in the RC.

The following points were highlighted in these reports: :

The samples collected had an average seed weight of between 100 and 200 g. First, the species *Callosobruchus maculatus* was identified on the *Cajuns cajan* pea variety from the department of Madingou. Secondly, of the three samples collected (two from Mouyondi and one from Loudima) three species *C. maculatus*, *Callosobruchus rhodesianus* and last *Bruchidius atrolineatus* were identified avec les proportions de two thirds, two thirds and one third respectively. In another report by Delobel in 1984, he states that *C. maculatus* is the most common bruchid species on all the leguminous varieties grown in the department of Bouenza, only the *Psophocarpus tetragonolobus* variety has proved resistant to its development (Delobel 1984).

A recent study has been published on the diversity of cytochrome oxidase 1 insect pests of peas and beans. The method used is phylogenetic reconstruction (Roy et al. 2023). In our study we are interested in the biodiversity of insect pests on two pea varieties. To achieve this goal, we will use the genetic distance method with the 12S rRNA marker. We believe that the use of markers other than COI would enrich the database, making multilocus barcoding more accessible and speeding up the biodiversity inventory. Our study focused on the identification by the 12S rRNA fragment of the bruchids that attack two pea varieties in RC: *Cajanus cajan* and *Vigna unguiculata* subsp. *cylindrica*. These two varieties are widely consumed by the Congolese population.

Methods

Bruchids survey

A random sampling of different populations of bruchid species was carried out from April 2019 to August 2019 in the departments of Niari and Bouenza (Table 1, Fig. 1). In this study 80 individuals were considered. The random sampling is a method of randomly and independently taking 'n' sample units from a population of 'N' elements. The samples are distributed randomly. Each point in the space studied therefore has an equal chance of being sampled. The data collected in this way are not biased. For this study, eight plots of pea fields were selected at each locality with a minimum distance of 4 km between plots. Twelve yellow sticking traps were set up on each plot. Traps were checked once per week, and bruchid-like beetles were collected manually. Individuals were collected from seed-baited traps and crop storage areas. The samples were placed in closed plastic containers with a paper towel soaked in ethyl acetate for 24 h. This procedure prevents the specimens from becoming rigid. Specimens were then labelled according to plot location and preserved in 70% ethanol for morphological analysis and 95% ethanol for molecular analysis.

Morphological analyses

Each bruchid specimen was placed on a Petri dish lid and observed under a Leica MZ6 modular stereomicroscope (Leica Microsystems GmbH, Wetzla, Germany). Specimens were classified using species identification keys (Kingsolver et al. 2017).

Molecular analyses

DNA extraction

Total genomic DNA was extracted from 20 individuals, 10 and 10 from Bouenza and Niari. The total DNA was purified using the spin-column protocol (Qiagen, Hilden, Germany). In this protocol, insects are frozen in liquid nitrogen and then ground with a mortar and pestle or disposable microtube pestle, or homogenised using the Tissue Ruptor (Qiagen), an equivalent electric homogeniser. Samples were then processed using the DNeasy® Blood & Tissue Kit (Qiagen). DNA elution was performed in Tris-acetate-EDTA (TAE) instead of water.

DNA amplification and sequencing

The 12S rRNA mitochondrial fragments were amplified and sequenced. The primers used for amplification of the 12S rRNA gene were 12S-F 5'-TACTATGTTACGACTTAT-3' and 12S-R 5'-AAACTAGGATTAGATACCC-3' (Tixier et al. 2006; Okassa et al. 2009, 2010; M Hernández-Triana et al. 2019). The amplification reactions for 12S fragments were carried out with Dr. MAX DNA Polymerase (Doctor protein INC, Korea, cat.no.: DR00302). A Bio-Rad DNA Engine Tetrad 2 Peltier Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used for PCR amplification. To amplify the 12S rRNA fragments, samples were denatured at 95°C for 1 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 40°C for 30 s, extension at 72°C for 1 min, and a final step at 72°C for 5 min. After

amplification, 5 µL of the PCR reaction was analysed by 1% agarose gel electrophoresis running for 20 min at 300 V, 200 A, and 2 µL of DNA loaded. PCR products were purified using a Multiscreen filter plate (MilliporeSigma, Burlington, MA, USA). The amplified 12S rRNA fragments containing visible and single bands were directly sequenced using an ABI PRISM 3730XL Analyser (96 capillary type) with a BigDye® Terminator ver. 3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA)

DNA Blast

The Geneious Prime 2019.2 (Raupach et al. 2010) was used to correct and assemble the raw data into sequence contigs. The assignment of each query sequence was performed using the local basic alignment search tool (MegaBLAST) on an external NCBI database. An aligned set of potentially related sequences were obtained, ranked according to their similarity. This similarity takes into account different parameters, with the most relevant being the bit score, E-value, percent identity, and grade (Okassa et al. 2020; Dibangou et al. 2021).

Sequence variation and divergence analysis

To the obtained sequence data set were added three sequences of *C. maculatus*, with two sequence de *C. maculatus* from the department of Bouenza accidentally identified on the bean (Dibangou et al. 2021) and one sequence from the country of Senegal (Kergoat et al. 2005). In last, one sequence of *Bruchus pisorum* from the country of Italy (Kergoat et al. 2007) was considered as outgroup species.

The sequences were aligned using the MUSCLE alignment procedure with gap penalties, iteration and clustering method (Edgar 2004). An analysis zone was defined from sites 17 to 402 in order to consider only the same number of character states for all the sequences studied, either 386 sites. The best sequence evolution model of 24 models was determined using Molecular Evolution Genetics Analysis (MEGA) version 11 (Tamura et al. 2021) with maximum likelihood (Kumar et al. 2016). Models with the lowest Bayesian Information Criterion (BIC) scores were considered to best describe the substitution pattern. The corrected Akaike information criterion, maximum likelihood value (ML), and number of parameters (including branch length) were included in each model (Nei et al. 2000). The non-uniformity of evolutionary rates among sites were modelled using a discrete Gamma distribution (+ G) with 5 rated categories, assuming that a certain fraction of the sites are evolutionarily invariable (+ I). Whenever applicable, estimates of the gamma shape parameter and/or the estimated fraction of invariant sites were reported.

MEGA 11 was employed also to analyze nucleotide composition, conserved sites, variable sites, singleton sites and parsimony-informative sites (Tamura et al. 2021).

At last, analyses estimates of evolutionary divergence between sequences were conducted using the Tamura 3-parameter model (Tamura 1992). The rate variation among sites was modeled with a gamma distribution (shape parameter = 1).

Genetic diversity

Evaluation of the number of haplotypes and haplotype diversity between all populations considered were performed using DnaSP software version 6.12 (Librado and Rozas 2009).

Results and discussion

Morphological analyses

The use of an identification key allowed us to assign each individual to a specific rank (Kingsolver et al. 2017). Individuals belonging to *Callosobruchus maculatus* because they have the following two characteristics 1) hind femur with a spine or denticles on both latero ventral and mesoventral carinae and 2) Elytral stria originating at basal margin and lacking denticles or gibbosity. A second species belonging to the Curculionidae family was identified, she is characterised by the presence of a rostrum extended beyond the eyes, with mouth parts situated at its apex. This morphological trait is one of the most typical features of the family of Curculionidae.

Molecular analyses

Sequence variation

Fragments of 423 bp were aligned for 12S rRNA and contained 344 conserved, 76 variables, 31 parsimony-informative, and 40 singleton sites. All new sequences herein obtained have been deposited in GenBank and are shown in Table 2.

Genetic distances

Mean genetic Tamura 3 parameter (substitutions models) and evolutionary rates among sites (+ G = discrete gamma) distances (minimum and maximum values) within and between all populations studied with outgroup species *B. pisorum* for 12S rRNA are shown in Table 2

First, the genetic distances between the populations of *C. maculatus* originating from different localities in Congo were very small and had little variation (0–0.8% with a mean of 0.2%). This low genetic variability can be explained by host plants (pea variety) occurring in similar climatic conditions (temperature and hygrometry) between Bouenza and Niari. In addition, little genetic divergence was found between *C. maculatus* collected from pea and *C. maculatus* collected from bean (0–1.5% with an mean of 0.6%). The genetic distances between *C. maculatus* populations originating from the Republic of Congo and those from Senegal were greater and varied between 3.3–4.2%, which corresponds to interspecific variation. For example, for *Acanthocseslides obtectus*, which are in the same family, the mean intraspecific variation was 0 and 0.62% for specimens from Bouenza and Niari. The host plant and climatic differences between these two countries could explain this variability.

Second, the genetic distances between *C. maculatus* populations and the population comprising individual 26 belonging to the Curculionidae family were 12.7%, showing interspecific variation at a

threshold greater than 3%. These results confirm our morphological observations and support the presence of another important biological pest, belonging to an other family.

A last, a comparison of the DNA sequence of *B. pisorum* from Italy, retrieved from GenBank, was performed. The genetic distances between the different populations of *C. maculatus* and *B. pisorum* varied from 13.9 to 16.4%, and corresponded to interspecific variation .

Except for the case of *C. maculatus* from Senegal,, the assignment of each individual adhered to two principles of the barcode: that 1) intraspecific variation was less than interspecific variation and 2) the absence of overlap between intra- and interspecific variation (presence of a gap) (Hebert et al. 2003).

Haplotypic diversity

Nine haplotypes were identified in the study species (Table 2), with significant haplotype diversity ($H_d = 0.8182$). With regards to our sample size, the number of distinct haplotypes and the degree of haplotype diversity showed a high degree of haplotype differentiation in the beetles in our dataset. The results of this study were similar to those obtained by other authors hypothesised, based on their results, that natural selection, particularly frequency dependent selection, could play a role in the differentiation of mitochondrial genetic variation by maintaining within *C. maculatus* populations a variation of mitochondrial DNA haplotypes (Tuda et al. 2014).

Conclusion

This study has demonstrated the potential role of integrative taxonomy (Morphological and molecular analyses) in classifying important pest species belonging to the Chrysomelidae family and confirmed their presence in domestic pea varieties of the Republic of Congo. This tool allowed us to identify the species *C. maculatus*, which is the most dominant pest on *C. cajan* and *V. u. cylindrica* varieties in agricultural fields and storage systems in Niari and Bouenza. In view of the dispersal capacity and voracity of these pests, implementing an effective and environmentally friendly integrated biological control system is urgent. In addition, in contrast to the report published by to Delobel in 1984 we did not identify *Callosobruchus rhodesianus* and *Bruchidius atrolineatus* were not identified in our study. In order to have a complete overview of the biodiversity of Bruchidia on pea, it would be necessary in a future study to expand the sample area.

Declarations

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

1. Agnarsson I, Kuntner M (2007) Taxonomy in a Changing World: Seeking Solutions for a Science in Crisis. *Syst Biol* 56:531–539
2. Ashfaq M, Hebert PDN (2016) DNA barcodes for bio-surveillance: regulated and economically important arthropod plant pests. *Genome* 59:933–945
3. Bakhoun MT, Sarr M, Fall AG, Huber K, Fall M, Sembène M, Seck MT, Labuschagne K, Gardès L, Ciss M, Gimonneau G, Bouyer J, Baldet T, Garros C (2018) DNA barcoding and molecular identification of field-collected *Culicoides* larvae in the Niayes area of Senegal. *Parasit Vectors* 11:615
4. Borowiec L (1987) The genera of seed-beetles (Coleoptera, Bruchidae). *Pol Pismo Entomol* 57:2–207
5. Boxall RA (1986) A critical review of the methodology for assessing farm-level grain losses after harvest. Tropical Development and Research Institute, London
6. Boykin LM, Armstrong KF, Kubatko L, De Barro P (2012) Species delimitation and global biosecurity. *Evol Bioinforma Online* 8:1–37
7. Delobel A (1984) Les Coléoptères des denrées stockées en République Populaire du Congo (liste provisoire). 28 p. multigr.
8. Delobel A (2000) Comportements de ponte chez les Caryedon (Olivier). Laboratoire ITA/IRD de Protection des Stocks,
9. Delobel A, Epouna-Mouinga S (1984) Les structures paysannes de stockage en République Populaire du Congo: pré-enquête dans la région de la Bouenza.
10. Dibangou V, Okassa MBM, Mazikou GFM, Lenga A (2021) Molecular characterization of pests (Chrysomelidae: Bruchinae) of beans (*Phaseolus vulgaris*) in the Republic of Congo. *Afr Zool* 56:35–43
11. Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5:113
12. Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. *Proc Biol Sci* 270:313–321
13. Huignard J, Glitho I, Monge J-P, Régnault-Roger C (2011) Insectes ravageurs des graines de légumineuses - Biologie des bruchinae et lutte raisonnée en Afrique.
14. Jeyaprakash A, Hoy MA (2002) Mitochondrial 12S rRNA sequences used to design a molecular ladder assay to identify six commercially available phytoseiids (Acari: Phytoseiidae). *Biol Control* 25:136–142
15. Jeyaprakash A, Hoy MA (2007) The mitochondrial genome of the predatory mite *Metaseiulus occidentalis* (Arthropoda: Chelicerata: Acari: Phytoseiidae) is unexpectedly large and contains

several novel features. *Gene* 391:264–274

16. Johnson CD (1981) Seed beetle host specificity and the systematics of the Leguminosae. *Adv Legume Syst*
17. Keneni G, Bekele E, Getu E, Imtiaz M, Damte T, Mulatu B, Dagne K (2011) Breeding Food Legumes for Resistance to Storage Insect Pests: Potential and Limitations. *Sustainability* 3:1399–1415
18. Kergoat GJ, Delobel A, Fédière G, Rü BL, Silvain J-F (2005) Both host-plant phylogeny and chemistry have shaped the African seed-beetle radiation. *Mol Phylogenet Evol* 35:602–611
19. Kergoat GJ, Silvain J-F, Delobel A, Tuda M, Anton K-W (2007) Defining the limits of taxonomic conservatism in host–plant use for phytophagous insects: Molecular systematics and evolution of host–plant associations in the seed-beetle genus *Bruchus* Linnaeus (Coleoptera: Chrysomelidae: Bruchinae). *Mol Phylogenet Evol* 43:251–269
20. Kingsolver J, Tuñón JB, Nápoles J, Thomas M (2017) Bruchidae of Chile (Insecta: Coleoptera). *Insecta Mundi*
21. Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol* 33:1870–1874
22. Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451–1452
23. M Hernández-Triana L, A Brugman V, I Nikolova N, Ignacio Ruiz-Arrondo null, Barrero E, Thorne L, Fernández de Marco M, Krüger A, Lumley S, Johnson N, R Fooks A (2019) DNA barcoding of British mosquitoes (Diptera, Culicidae) to support species identification, discovery of cryptic genetic diversity and monitoring invasive species. *ZooKeys* 832:57–76
24. Magoga G, Sahin DC, Fontaneto D, Montagna M (2018) Barcoding of Chrysomelidae of Euro-Mediterranean area: efficiency and problematic species. *Sci Rep* 8:13398
25. Nei P of PGB of the C for D and PGM, Nei M, Kumar S, Nei EPP of BM (2000) *Molecular Evolution and Phylogenetics*. Oxford University Press,
26. Okassa M, Tixier M-S, Cheval B, Kreiter S (2009) Molecular and morphological evidence for a new species status within the genus *Euseius* (Acari: Phytoseiidae). *Can J Zool* 87:689–698
27. Okassa M, Tixier M-S, Kreiter S (2010) Morphological and molecular diagnostics of *Phytoseiulus persimilis* and *Phytoseiulus macropilis* (Acari: Phytoseiidae). *Exp Appl Acarol* 52:291–303
28. Okassa MBM, Ntabi DM, Lenga A (2020) Morphological and molecular identification of specimens in the genus *Euseius* (Acari: Phytoseiidae) from the Republic of Congo. *Zootaxa* 4768:479–498
29. Raupach MJ, Astrin JJ, Hannig K, Peters MK, Stoeckle MY, Wägele J-W (2010) Molecular species identification of Central European ground beetles (Coleoptera: Carabidae) using nuclear rDNA expansion segments and DNA barcodes. *Front Zool* 7:26
30. Roy V, Mpika J, Kergoat G, Mboussy GFT, Attibayeba A (2023) Diversity of insect pests of common bean and pigeon pea in the Republic of Congo revealed by DNA barcoding. *Afr Entomol* 31:

31. Tamura K (1992) Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. *Mol Biol Evol* 9:678–687
32. Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol Biol Evol* 38:3022–3027
33. Tixier M-S, Kreiter S, Barbar Z, Ragusa S, Cheval B (2006) Status of two cryptic species, *Typhlodromus exhilaratus* Ragusa and *Typhlodromus phialatus* Athias-Henriot (Acari: Phytoseiidae): consequences for taxonomy. *Zool Scr* 35:115–122
34. Tuda M, Kagoshima K, Toquenaga Y, Arnqvist G (2014) Global Genetic Differentiation in a Cosmopolitan Pest of Stored Beans: Effects of Geography, Host-Plant Usage and Anthropogenic Factors. *PLOS ONE* 9:e106268

Tables

Table 1 Sampling locations of different populations of species considered in this study.

Department	Locality	Sites	Latitude	Longitude
Bouenza	Boko songho	1	-4.44107	13.6208
Bouenza	Kala-Boutoto	2	-4.1629	13.71094
Niari	Kikassa 2	3	-4.29508	12.7739
Niari	Louvakou	4	-3.967644	12.593317
Niari	Kimongo	4	-4.483533	12.947302
Bouenza	Madingou	5	-4.163371	13.553048
Bouenza	Madingou	5	-4.163371	13.553048
Bouenza	Mouyouenzi	6	-3.890046	13.958513
Bouenza	Yenge	7	-4.41503	13.6673

Table 2. Characteristics of individuals and species considered in the molecular analysis with their accession numbers. The sequences directly retrieved from Genbank database have an asterisk. RC: Republic of Congo.

Family	Genus	Specie	Host plant	Isolate	Haplotype	Country	Sites	Acession number
Chrysomelidae	<i>Callosobruchus</i>	<i>maculatus</i>	<i>Cajanus cajan</i>	7	5	RC	6	MN396176
Chrysomelidae	<i>Callosobruchus</i>	<i>maculatus</i>	<i>Cajanus cajan</i>	8	5	RC	5	MN396177
Chrysomelidae	<i>Callosobruchus</i>	<i>maculatus</i>	<i>Cajanus cajan</i>	11	6	RC	5	MN396178
Chrysomelidae	<i>Callosobruchus</i>	<i>maculatus</i>	<i>Vigna unguiculata</i> subsp. <i>cylindrica</i> (L.).	27	5	RC	4	MN396180
Chrysomelidae	<i>Callosobruchus</i>	<i>maculatus</i>	<i>Vigna unguiculata</i> subsp. <i>cylindrica</i> (L.).	28	4	RC	1	MN396181
Chrysomelidae	<i>Callosobruchus</i>	<i>maculatus</i>	<i>Vigna unguiculata</i> subsp. <i>cylindrica</i> (L.).	30	3	RC	3	MN396182
Curculionidae	<i>Weevil</i>	<i>sp</i>	<i>Vigna unguiculata</i> subsp. <i>cylindrica</i> (L.).	26	7	RC		MN396179
Chrysomelidae	<i>Callosobruchus</i>	<i>maculatus</i>	<i>Phaseolus vulgaris</i>	19	1	RC		MN420803*
Chrysomelidae	<i>Callosobruchus</i>	<i>maculatus</i>	<i>Phaseolus vulgaris</i>	23	2	RC		MN420806*
Chrysomelidae	<i>Callosobruchus</i>	<i>maculatus</i>	<i>Vigna unguiculata</i>		8	Senegal		AY625320*
Chrysomelidae	<i>Bruchus</i>	<i>pisorum</i>	-		9	Italy		DQ307631*

Table 3. Genetic distances between and within *Callosobruchus maculatus* from Republic of Congo and Senegal, individual belonging to the family of Curculionidae and outgroup species *Bruchus* species for the one molecular marker used 12S rRNA.

12S rRNA	<i>Callosobruchus maculatus</i> RC	<i>Callosobruchus maculatus</i> Senegal	<i>Bruchus pisorum</i> Italy
<i>Callosobruchus maculatus</i> RC	0.0045(0-0.015)		
<i>Callosobruchus maculatus</i> Senegal	0.037(0.033-0.042)	-	-
Curculionidea RC	0.11(0.10-0.122)	0.127	0.149
<i>Bruchus pisorum</i> Italy	0.15(0.139-0.164)	0.149	-

Figures

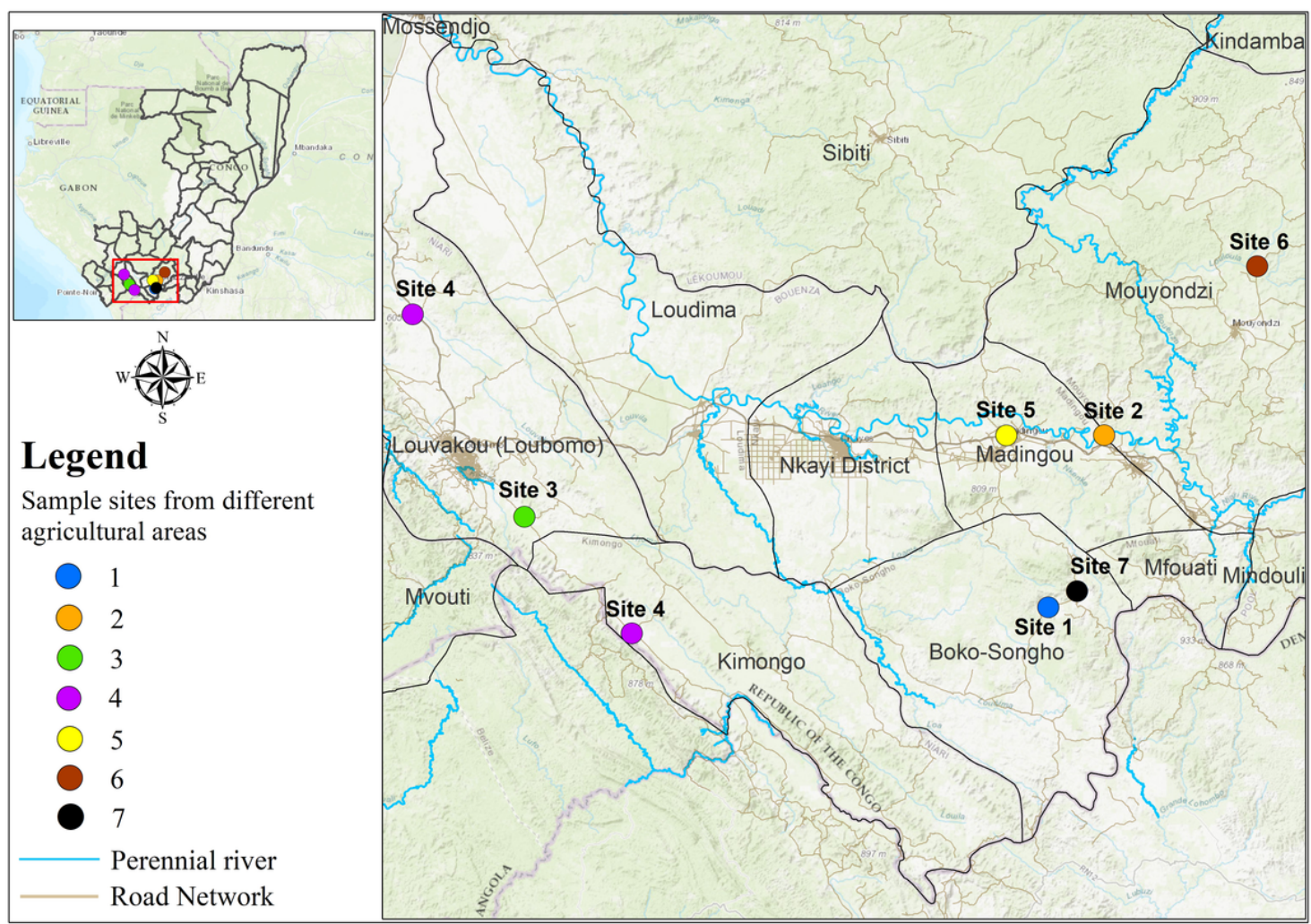


Figure 1

Sampling locations of different populations of specimens considered in this study. Site 1 blue, Site 2 orange, Site 3 green, Site 4 purple, Site 5 yellow, Site 6 brown, site 7 black