Vol. 23(10), pp.280-286, October 2024
DOI: 10.5897/AJB2024.17694
Article Number: DBD3BF972650
ISSN: 1684-5315

http://www.academicjournals.org/AJB

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biotechnology

Full Length Research Paper

Identification and molecular characterization of *Caryedon furcatus* (Anton & Delobel), the main pest of *Senegalia macrostachya* (Reichenb. ex DC. Kyal & Boatwr) seeds in storage in Burkina Faso

Larissa Dioma[1]*, Jean Christophe Koussoubé[1,2], Mbacké Sembène[3], Antoine Sanon[1] and Zakaria Ilboudo[1]

[1]Laboratoire d'Entomologie Fondamentale et Appliquée, Université Joseph KI ZERBO, 06 BP 9499 Ouagadougou 06, Burkina Faso.
[2]Centre Universitaire de Tenkodogo, Université Thomas SANKARA, 12 BP 417 Ouagadougou 12, Saaba, Burkina Faso.
[3]Département de Biologie Animale, Faculté des Sciences et Techniques, Université Cheikh Anta Diop, B.P. 5005 Dakar, Sénégal.

Received 2 August, 2024; Accepted 13 September, 2024

In Burkina Faso, seeds of *Senegalia macrostachya* (Rchb. Ex DC.) Kyal. & Boatwr. during storage are attacked by a pest morphologically identified as *Caryedon mauritanicus* Auct. It was described for the first time in 2004 under a new name '*Caryedon furcatus*' by Anton and Delobel. Thus, *C. mauritanicus* is now considered to be *C. furcatus*. In the present study, for the first time, genetic characterization of *C. furcatus* was carried out to determine its genetic identity in order to assess whether it is evolutionarily similar or related to *C. mauritanicus*. The pest's 12S gene was partially sequenced after extraction and amplification by Polymerase Chain Reaction. The percentage identity and variability of genetic parameters between *C. furcatus* and *C. mauritanicus* sequences were determined. A very high percentage of identity, ranging from 93.10 to 99.67% with respect to the *C. mauritanicus* species was obtained. The results also showed that eleven of the 208 sites were polymorphic. The values for nucleotide composition show a predominance of Adenine and Thymine (76.32%) when compared with Guanine and Cytosine (23.68%). *C. furcatus* has high haplotypic diversity (0.717) and low nucleotide diversity (0.006). The similarity percentage obtained shows that *C. furcatus* is genetically identical to *C. mauritanicus*.

Key words: *Senegalia macrostachya*, *Caryedon furcatus*, *Caryedon mauritanicus*, molecular identification, genetic identity.

INTRODUCTION

In Burkina Faso, a store pest morphologically similar to *Caryedon furcatus* emerges from *Senegalia macrostachya* seeds during storage (Yamkoulga et al., 2021a, b). *Senegalia macrostachya* holds a prominent place among these undomesticated wild food legumes due to the multiple benefits its seeds provide. These seeds contribute significantly to improving the population's diet, generates substantial income for rural populations and also hold potential in the pharmaceutical industry (Hama-Ba et al., 2017; Msika et al., 2017; Guissou et al., 2020). This species was recorded as *Caryedon mauritanicus* Decelle (Nongonierma, 1978; Decelle, 1979;

Varaigne-Labeyrie and Labeyrie, 1981), then as *C. mauritanicus* sensu Decelle (1979) (Anton, 1994) and *C. mauritanicus* Auct. (Silvain and Delobel, 1998; Delobel et al., 2000). The description of *C. mauritanicus* has never been published, so for the first time, Anton and Delobel (2004), decided to describe it under a new name "*C. furcatus*" to avoid any confusion. *C. mauritanicus* is now considered to be *C. furcatus*. However, more in-depth studies using new identification techniques are essential to confirm or refute the identity of these two species. Genetic identification, using molecular biology and bioinformatics tools and techniques, is a precise and effective approach for discriminating between species at all stages of their development. There are no data in the literature referring to the molecular identification of *C. furcatus*. However, *C. mauritanicus* has been identified morphologically as *C. furcatus* (Anton and Delobel, 2004), but this has not been verified molecularly. The work of Silvain and Delobel in 1998 provided a sequence of the 12S gene of *C. mauritanicus*, which is the only *C. maritanicus* gene available. So, on the basis of these data, the 12S gene (MT-RNR1) was chosen in this study as a marker to discriminate between the population of two insects infesting *S. macrostachya* seeds in storage, in order to assess their similarity or evolutionary relationship. The background to the present study, aims to carry out a molecular identification of the species and to characterize the genetic variability that exists within the species using a set of parameters.

Animal mtDNA has long been used as a marker of choice for population genetics, phylogeography and phylogenetic studies (Avise et al., 1987; Simon et al., 1994). In fact, mtDNA is present in cells in haploid form, reproduction is exclusively clonal (Hagelberg et al., 1999; Avise, 1991) and transmission is essentially maternal (Birky, 2001). 12S (MT-RNR1) is a ribosomal RNA from the small subunit of the ribosome encoded by the mitochondrial genome, the sequences of which are widely used for phylogeny studies. From the nucleotide sequences of *C. furcatus* obtained and those of *C. mauritanicus* available in the National Center for Biotechnology Information (NCBI), the percentage of similarity was determined and the genetic identity of *C. furcatus* was determined.

MATERIALS AND METHODS

Biological material

Specimens of *C. furcatus* used in the study were obtained from *S. macrostachya* seeds collected from spontaneous vegetation in "Barma" (Figure 1) and kept for breeding in the Laboratory of Fundamental and Applied Entomology, University Joseph KI-ZERBO.

At emergence, forty (40) individuals of *C. furcatus* were isolated and placed individually in coded Eppendorf tubes containing 96% alcohol and stored at -20°C for molecular analysis.

DNA extraction, polymerase chain reaction (PCR) and sequencing

The total genome of each individual was extracted using the Standard method of the Zymo Research Kit. The insects were first dissected and then carefully crushed, without the abdominal part. PCR was performed for the 12S mitochondrial gene using the "Taq®DNA Polymerase with Standard Taq Buffer" kit and primers F (5'-AAGAGCGACGGGCGATGTGT-3') and R (5'-AAACTAGGATTAGATACCCTATTAT-3') (Silvain and Delobel, 1998) covering a region of 355 bp. The volume of the PCR reaction mixture was 25 µl and included 1 µl of DNA extract, 2.5 µl of 10x buffer, 1.5 µl of $MgCl_2$, 0.5 µl of dNTP, 1.25 µl of each of the two primers, 0.1 µl of Taq polymerase, and 16.9 µl of sterile water. The PCR reaction was performed using an Eppendorf thermal cycler under the following conditions: an initial denaturation step at 95°C for 4 min followed by 35 amplification cycles (95°C for 30 s, 52°C for 30 s, 72°C for 1 min, and final elongation at 72°C for 10 min). The amplification products were then analyzed by electrophoresis on a 2% agarose gel in 0.5 × TAE buffer at 100 V for 30 min.

Sequencing reactions were performed in an MJ Research PTC-225 Peltier thermal cycler using ABIPRISM BigDye TM Terminator Cycle kits. Each sample was sequenced using the sense primer with 10 µl of PCR products. Fluorescent fragments were purified using the BigDye Xterminator purification protocol. Samples were suspended in distilled water and subjected to electrophoresis on an ABI 3730xl sequencer (Applied Biosystems).

Analysis of genomic sequences

The sequences obtained from the 40 samples were compared by alignment with other nucleotide sequences available in NCBI[1] (Nucleotide Center for Biotechnology Information) using BLASTn tool version 2.14.1+[2]. A percentage of identity between sequences greater than 70% meant that they were homologous. The sequences obtained were corrected and cleaned up manually by referring to the chromatograms. They were then aligned with BioEdit 7.0.8.0 (Hall, 1999) using the Clustal-W algorithm (Thompson et al., 1994).

The genetic diversity of *C. furcatus* was analyzed within the population by determining various parameters, such as the number of polymorphic sites, the number of informative sites, the number of haplotypes, haplotypic diversity (Hd), nucleotide diversity (π) (Nei and Tajima, 1981; Nei, 1987), and the average number of nucleotide differences (k) were brought out under Dnasp, version 5.10.01 (Rozas et al., 2017). The ratio between transitions and transversions as well as the nucleotide frequency were also calculated in the same software using the Pattern substitution test. The haplotype network was constructed with NETWORK ver. 10.2.0.0 using the Median-Joining method (Bandelt et al., 1999). The construction of this network is based on the theory of coalescence (Kingman, 2000), which consists of a retrospective

*Corresponding author. E-mail: bazielarissa1@gmail.com. Tel: 00226 76863761.

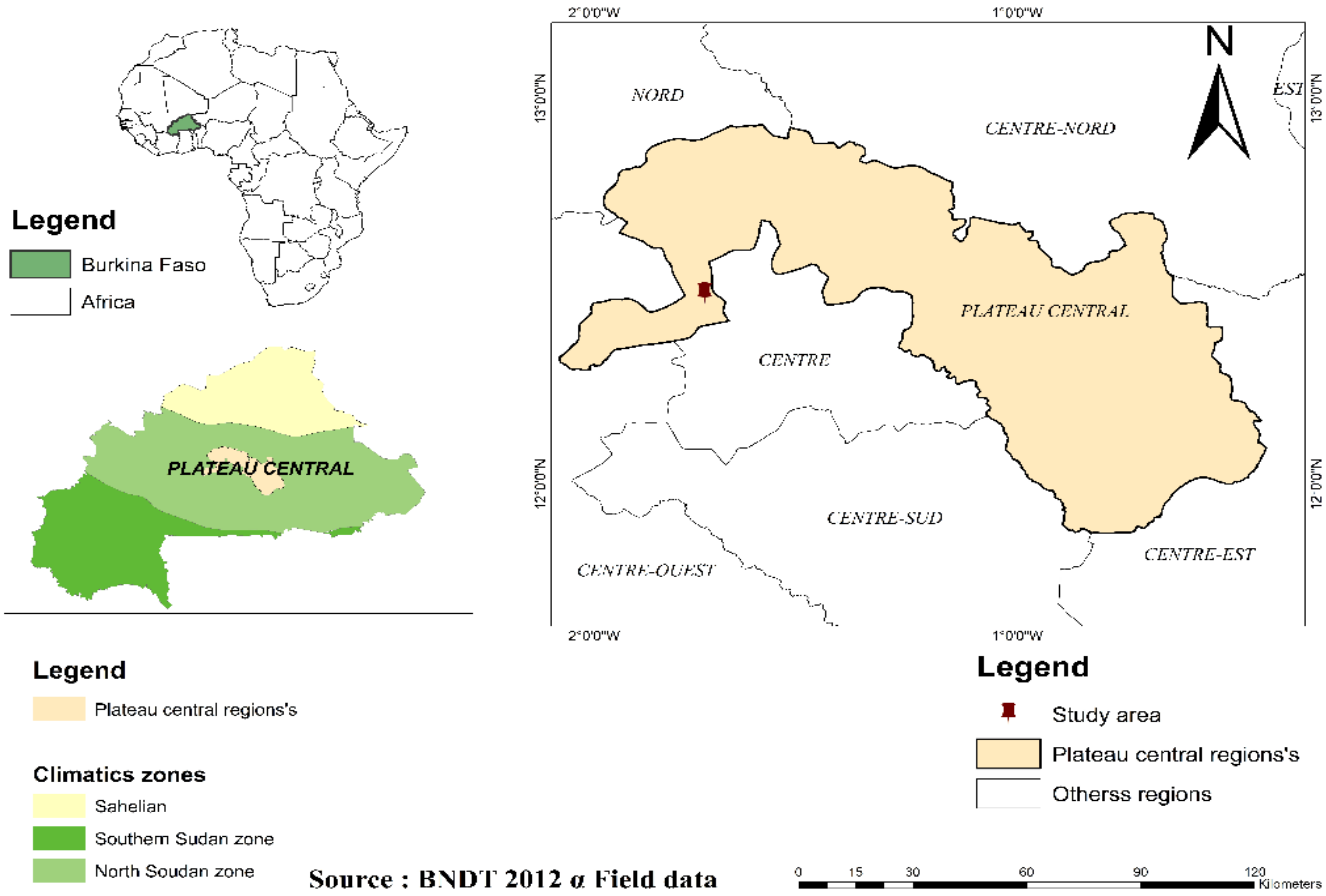

Figure 1. Map of the location site.

approach that mathematically describes the process of binary fusion of all the genealogical lineages of a sample of genes back to their closest common ancestor.

RESULTS

Genetic identity of *C. furcatus*

Analysis of C. furcatus sequences

After the blast, all the individuals had a very high percentage of identity, varying between 93.10 and 99.67% with respect to *C. mauritanicus*, with highly significant E-values (Table 1).

Molecular characteristics of C. furcatus

Of the forty (40) nucleotide sequences of *C. furcatus* 12S gene obtained after sequencing, 39 were well aligned at 208 bp. The nucleotide sequence of individual Cf36 was removed due to the lack of clarity of the chromatogram. Genetic polymorphism values are shown in Table 2. Eleven polymorphic sites and a total of 11 mutations were found. Values for nucleotide composition give 43.07% thymine (T), 33.25% adenine (A), 14.94% cytosine (C), and 8.74% guanine (G). This leads to a predominance of A and T (76.32%) compared with G and C (23.68%). *C. furcatus* has high haplotypic diversity (Hd=0.717) and low nucleotide diversity (π= 0.006) (Table 2). Analyses revealed significantly negative Tajima D and Fu Fs values for demographic parameters (Table 2).

Haplotypes and haplotype network

Eleven haplotypes were found. Haplotype H3 is in the majority, comprising 20 individuals. It underwent mutational steps to give haplotypes H1, H2, H4, H6, H7 and H10. The greatest number of mutational steps was observed between the majority haplotype and haplotype H6 (208 mutational steps). In addition, six private haplotypes were noted: H4 (Cf14), H5 (Cf23), H6 (Cf32), H7 (Cf38), H9 (Cf12), and H11 (Cf31) (Figure 2).

Table 1. Summary of Blast results for each individual.

Code	Similar species name	Identity (%)	*E-value*	Accession number
Cf1	*Caryedon mauritanicus*	99.67	3e-148	AF004126.1
Cf2	*Caryedon mauritanicus*	99.67	9e-149	AF004126.1
Cf3	*Caryedon mauritanicus*	99.67	9e-149	AF004126.1
Cf4	*Caryedon mauritanicus*	97.72	2e-139	AF004126.1
Cf5	*Caryedon mauritanicus*	93.10	2e-113	AF004126.1
Cf6	*Caryedon mauritanicus*	94.83	2e-112	AF004126.1
Cf7	*Caryedon mauritanicus*	98.56	7e-131	AF004126.1
Cf8	*Caryedon mauritanicus*	96.94	2e-131	AF004126.1
Cf9	*Caryedon mauritanicus*	93.53	3e-116	AF004126.1
Cf10	*Caryedon mauritanicus*	95.85	7e-125	AF004126.1
Cf11	*Caryedon mauritanicus*	99.01	2e-144	AF004126.1
Cf12	*Caryedon mauritanicus*	90.77	1e-90	AF004126.1
Cf13	*Caryedon mauritanicus*	97.10	3e-135	AF004126.1
Cf14	*Caryedon mauritanicus*	95.71	6e-88	AF004126.1
Cf15	*Caryedon mauritanicus*	96.10	3e-130	AF004126.1
Cf16	*Caryedon mauritanicus*	96.42	3e-129	AF004126.1
Cf17	*Caryedon mauritanicus*	99.67	1e-147	AF004126.1
Cf18	*Caryedon mauritanicus*	96.69	2e-132	AF004126.1
Cf19	*Caryedon mauritanicus*	98.68	7e-144	AF004126.1
Cf20	*Caryedon mauritanicus*	97.65	2e-137	AF004126.1
Cf21	*Caryedon mauritanicus*	97.36	8e-137	AF004126.1
Cf22	*Caryedon mauritanicus*	98.36	1e-141	AF004126.1
Cf23	*Caryedon mauritanicus*	96.91	4e-128	AF004126.1
Cf24	*Caryedon mauritanicus*	94.16	1e-122	AF004126.1
Cf25	*Caryedon mauritanicus*	98.36	3e-142	AF004126.1
Cf26	*Caryedon mauritanicus*	94.10	1e-122	AF004126.1
Cf27	*Caryedon mauritanicus*	95.13	7e-125	AF004126.1
Cf28	*Caryedon mauritanicus*	98.02	2e-139	AF004126.1
Cf29	*Caryedon mauritanicus*	98.68	6e-145	AF004126.1
Cf30	*Caryedon mauritanicus*	96.44	5e-133	AF004126.1
Cf31	*Caryedon mauritanicus*	89.80	5e-101	AF004126.1
Cf32	*Caryedon mauritanicus*	93.93	1e-122	AF004126.1
Cf33	*Caryedon mauritanicus*	98.66	1e-140	AF004126.1
Cf34	*Caryedon mauritanicus*	97.02	4e-134	AF004126.1
Cf35	*Caryedon mauritanicus*	99.67	9e-149	AF004126.1
Cf36	*Caryedon mauritanicus*	98.76	7e-112	AF004126.1
Cf37	*Caryedon mauritanicus*	99.34	4e-147	AF004126.1
Cf38	*Caryedon mauritanicus*	99.01	6e-145	AF004126.1
Cf39	*Caryedon mauritanicus*	98.02	1e-141	AF004126.1
Cf40	*Caryedon mauritanicus*	94.84	5e-127	AF004126.1

Cf: *Caryedon furcatus.*

DISCUSSION

The aim of this study was to elucidate the molecular identification and genetic characterization of *C. furcatus*, the main pest associated with *S. macrostachya* seeds during storage. The individuals were sampled in Burkina Faso and the study was carried out using PCR-sequencing of the 12S gene. The blast results showed high percentages of similarity, varying between 93.10 and 99.67% between *C. furcatus* and *C. mauritanicus*. This would mean that *C. furcatus*, which is the subject of this study, is very close genetically to *C. mauritanicus*

Table 2. Genetic diversity and demographic change of *Caryedon furcatus.*

Genetic diversity parameter		Value
Sample size		39
Number of sites		208
Variable sites		11
Singleton variable sites		7
Variable sites in parsimony		4
Average number of nucleotide differences (k)		1.339
Nucleotide frequency	A+T	76.32
	C+G	23.68
Number of haplotypes (h)		11
Haplotypic diversity (Hd)		0.717±0.00510
Nucleotide diversity (π)		0.00644±0.0000012
Transition rate		67.7
Transversion rate		32.28
Mutation rate (R)		2.09
Total number of mutations (Eta)		11
Demographic parameter		
D de Tajima		-1,48032
Fs de Fu		-5,700

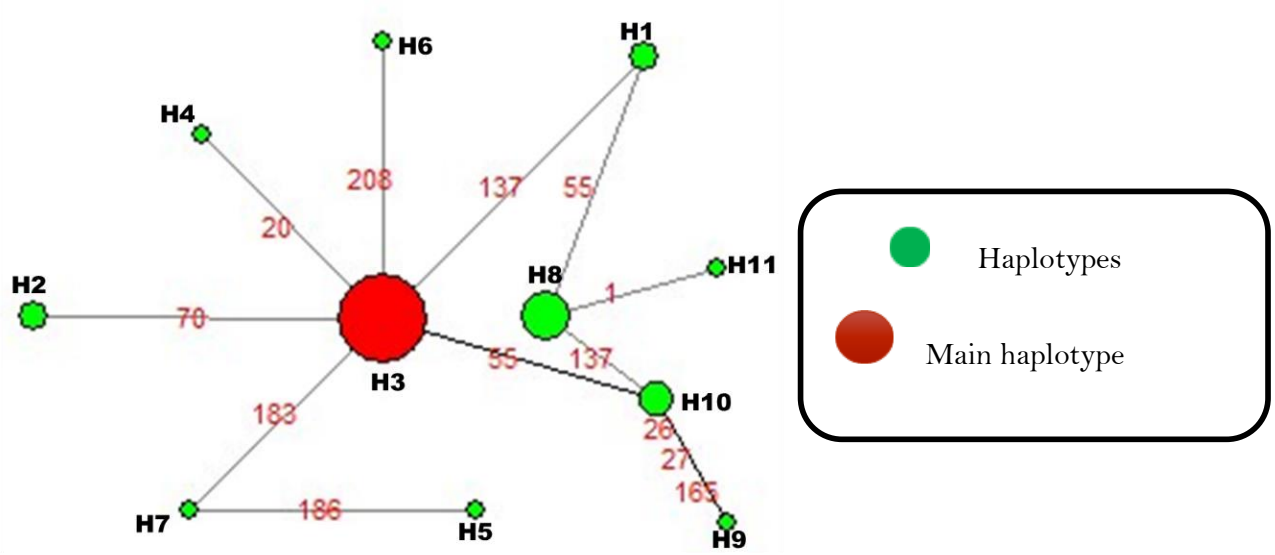

Figure 2. Haplotype network representing the evolutionary relationships between the different haplotypes of *Caryedon furcatus.*

indicated as a result that *C. furcatus* and *C. mauritanicus* are identical at the molecular level. With regard to the parameters of genetic variability, the results give a partial gene sequence 208 base pairs long. The number of polymorphic sites was 11, as was the number of mutations, indicating low variability in the *C. furcatus* 12S gene. This could be explained by the small size of the population, but also by the sampling area, which covered only one locality. Extending the sampling area and multiplying the number of individuals analyzed may

provide further information on the variability of this gene. Polymorphism analysis revealed high haplotypic diversity (hd=0.717) and low nucleotide diversity (π =0.006). This may correspond to rapid population growth from an ancestral population of low effective size, and an elapsed time sufficiently long to generate haplotypic variability through mutations, but insufficiently long to allow the accumulation of a large number of differences between sequences as suggested by Avise (2000) in his study of the history and formation of species. The overall nucleotide frequencies show that the percentage of A and T (76.32%) is much higher than the frequency of C and G (23.68%). This result suggests that the DNA molecule in *C. furcatus* is less dense, because G and C are heavier than A and T. In fact, G and C are linked by three hydrogen bonds, which are more stable than the two hydrogen bonds that link A and T. However, the low values for nucleotide diversity can be explained by the relatively low numbers due to the demographic instability of the target population. However, multiplying the number of individuals could provide greater precision (Telahigue et al., 2017). Demogenetic tests show demographic expansion, with Tajima's D and Fu's Fs values being significantly negative (Rogers and Harpending, 1992).

The relationships between the haplotypes of the individuals sampled, presented in the form of a haplotype network, enable us to make hypotheses about the evolutionary history of the populations analyzed. A star-shaped representation was obtained, with a very abundant haplotype and several derived haplotypes that differ from it by one or two mutational steps. This haplotype is present in all individuals, which may indicate the existence of recent genetic flows between them (Horne et al., 2008). It is probably the oldest; in fact, there is a positive correlation between the frequency and age of haplotypes, and it has probably been present in populations for a long time (Castelloe and Templeton, 1994; Posada and Crandall, 2001). Consequently, most new haplotypes derived from the most common haplotypes, meaning that the rarest variants correspond to the most recent mutations (Posada and Crandall, 2001). The hypothesis of rapid population expansion or fluctuations in population size with bottlenecks is also supported by this type of star network.

Conclusion

Knowledge of an insect pest is a crucial step in the search for effective and sustainable control methods. Through genetic characterization, the present study has shown that *C. furcatus* is identical to *C. mauritanicus*, which is known to be the main pest associated with *S. macrostachya* seeds during storage in Burkina Faso. This study represents a significant advance in the scientific context, as it now contributes to the precise molecular identification of *C. furcatus* based on the 12S gene. Based on these results, it is essential to carry out a more in-depth study by sampling in different localities and different agro-ecological zones of Burkina Faso, while increasing the number of individuals and studying as many genes in the mitochondrial and nuclear genome as possible.

ACKNOWLEDGEMENTS

This study was fully funded by the World Bank through the PAES (Programme d'Appui à l'Enseignement Supérieur) project. The authors thank GENOMICS laboratory and the Department of Animal Biology/Population Genetics at Cheikh Anta Diop University in Dakar and particular Dr Fatimata Mbaye for her supervision.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Anton KW (1994). The Bruchindae(Coleoptera) of Oman, with Descriptions of a New Genus and Two New Species. Fauna of Saudi Arabia 14:105-112.

Anton KW, Delobel A (2004). Description of five new species in the genus Caryedon Schoenherr, with a taxonomical note on C. angeri (Semenov) (Coleoptera: Bruchidae: Pachymerinae). Genus 15(1):65-90.

Avise JC (1991). Ten unorthodox perspectives on evolution prompted by comparative population genetic findings on mitochondrial DNA. Annual Review of Genetics 25(1):45-69.

Avise JC (2000). Phylogeography: The History and Formation of Species. Harvard University Press.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC (1987). Intraspecific phylogeography: The Mitochondrial DNA Bridge Between Population Genetics and Systematics. Annual Review of Ecology and Systematics 18:489-522.

Bandelt HJ, Forster P, Röhl A (1999). Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16(1):37-48.

Birky Jr CW (2001). The inheritance of genes in mitochondria and chloroplasts: laws, mechanisms, and models. Annual Review of Genetics 35(1):125-148.

Castelloe J, Templeton AR (1994). Root probabilities for intraspecifies genes trees under neutral coalescent theory. Molecular Phylogenetics and Evolution 3(2):102-113.

Decelle J (1979). Insects of Saudi Arabia: Coleoptera: Fam. Bruchidaele. Fauna of Saudi Arabia 1:318-330.

Delobel A,Tran M, Sembene M (2000). Influence du choix alimentaire sur la fécondité et le développement larvaire des Caryedon des légumineuses (Coleoptera : Bruchidae) au Sénégal. Annales de la Societe Entomologique de France 36(1):61-73.

Guissou AWDB, Parkouda C, Coulibaly KA, Traoré K, Ouboulbiga EB, Savadogo A (2020). Fermentation effet on the nutrient and antinutrient composition of Senegalia macrostachya and Parkia biglobosa seeds: Acomparative study. Food and Nutrition Sciences 11(7):726-740.

Hagelberg E, Goldman N, Lio P, Whelan S, Schiefenhöel W, Clegg JB,

Bowden DK (1999). Evidence for mitochondrial DNA recombination in a human population of Island Melanesia. Proceedings of the Royal Society of London Series B: Biological Sciences 266(1418):485-492.
Hall TA (1999). BioEdit: a user friendly biological seque. Nucleid Acids Symposium Series, 41(41):95-98.
Hama-Ba F, Siedogo M, Ouédraogo M, Dao A, Dicko HM, Diawara B (2017). Modalités de consommation et valeur nutritionnelle des légumineuses alimentaires au Burkina Faso. African Journal of Food, Agriculture, Nutrition and Development 17(4):12871–12888
Horne JB, Herwerden VL, Howard JC, Robertson DR (2008). High population connectivity across the Indo-Pacific: Congruent lack of phylogeographic structure in three reef fish congeners. Molecular Phylogenetics and Evolution 49(2):629-638.
Kingman JFC (2000). Origins of the coalescent: 1974-1982. Genetics 156(4):1461-1463.
Nei M (1987). Bibliography. Molecular Evolutionary Genetics. Columbia University Press 433-496
Nei M, Tajima F (1981). DNA polymorphism detectable by restriction endonucleases. Genetics 97(1):145-163.
Nongonierma A (1978). Contribution à l'étude biosystématique du genre Acacia Miller en Afrique occidentale. Thèse de Doctorat d'Etat, mention Sciences, Université de Dakar, Sénégal, 451 pp.
Posada D, Crandall KA (2001). Intraspecific gene genealogies: trees grafting into networks. Trends in Ecology & Evolution 16(1):37-45.
Rogers AR, Harpending H (1992). Population growth makes waves in the distribution of pairwise genetic differences. Molecular biology and evolution 9(3):552-569.
Rozas J, Ferrer-Mata A, Sanchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sanchez-Gracia A (2017). DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution 34(12):3299-3302.
Silvain JF, Delobel A (1998). Phylogeny of West African Caryedon (Coleoptera: Bruchidae): Congruence between Molecular and Morphological Data. Molecular Phylogenetics and Evolution 9(3):533-541.
Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87(6):651-701.
Telahigue K, Hajji T, Rabeh I, Cafsi E, Saavedra C (2017). Structure génétique et demographique du petoncle Flexopecten glaber de la lagune de Bizerte (Tunisie). Bulletin de l'Institut National Des Sciences et Technologies de La Mer de Salammbô 44:77-78. http://hdl.handle.net/1834/14926
Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22(22):4673-4680.
Varaigne-Labeyrie C, Labeyrie V (1981). First Data on Bruchidae Which Attack the Pods of Legumes in Upper Volta of Which Eight Species are Man Consumed. In: Labeyrie V, Ed., The Ecology of Bruchids Attacking Legumes (Pulses), Series Entomologica, Springer, Berlin, 19:83-96. https://doi.org/10.1007/978-94-017-3286-4 8
Yamkoulga M, Waongo A, Traoré F, Sawadogo L, Goergen G, Sanon A (2021a). Insect pests of stocks of Acacia macrostachya Reichenb and associated parasitoids in the province of Boulkiemde, central-western region of Burkina Faso. International Journal of Tropical Insect Science 41(4):2501-2510.
Yamkoulga M, Waongo A, Traoré F, Ilboudo Z, Sanon A (2021b). Some biological parameters of Caryedon furcatus (Anton & Delobel) (Coleoptera : Chrysomelidae), developing on the seeds of Senegalia macrostachya (Reichenb. Ex DC.) Kyal. & Boatwr. (Fabales : Mimosaceae), an edible wild legume in Burkina Faso. African Entomology 29(2):395-404.