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Molecular Characterization and Phylogenetic Analysis of Larger Grain Borer Prostephanus truncatus (Coleoptera: Bostrichidae) from Oyo State, Nigeria

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Abstract

The larger grain borer, *Prostephanus truncatus*, is an exotic insect pest of stored products in the tropics that poses a serious threat to food security. Indigenous to Central America, it has spread to many countries, including Nigeria, reducing the quantity and quality of stored maize and dried cassava, thereby reducing market value and farmers' incomes. Traditional taxonomic identification of this pest is based on laborious observations of morphological traits through a stereomicroscope, which can be unreliable due to overlapping features among closely related species. Accurate species identification is crucial for effective pest management, quarantine, and biosecurity programs. In this study, genetic analyses of *P. truncatus* were conducted on specimens collected from dried cassava chips from four purposively selected markets (Bodija-Ibadan, Sabo-Oyo, Oja-Agbe-Iseyin, and Sango-Saki) in Oyo State, Nigeria. A total of 28 adults (7 specimens per market) were collected and preserved in 70% alcohol for molecular analysis. DNA was extracted from whole insects using the Quick-DNA™ Tissue/Insect Miniprep kit. The target 28S gene (680 bp) was amplified according to standard methods, and all products were separated, purified, visualized under ultraviolet light, and documented. Successful amplification of the 28S gene was observed in all adult *P. truncatus* samples. The phylogeny revealed three major phenetic groups supported by bootstrap values ≥ 50%, and no significant genetic variations were observed. The genetically identified *P. truncatus* samples were monophyletic and highly identical (94–99%). These results provide valuable new information about *P. truncatus* and offer useful data for its detection and management.

Introduction

Prostephanus truncatus (Horn) (Coleoptera: Bostrichidae), widely known as the larger grain borer (LGB), is among the most economically important insect pests of stored maize and dried cassava in tropical and subtropical regions. Native to Central America and Mexico, the species was originally recognized as a wood-boring beetle before emerging as a major pest of stored agricultural commodities (Chittenden 1911; Hodges 1986). Since its accidental introduction into Africa through international trade and the movement of infested commodities, *P. truncatus* has become established in several African countries, where it has caused severe economic losses through the destruction of stored food products (Hodges 1986; EPPO 2013).

Unlike many primary storage pests that are restricted to cereal grains, *P. truncatus* has a broad host range and readily infests both stored maize and dried cassava chips. The insect bores extensively into the stored commodity, producing large amounts of powdery frass that significantly reduce the weight, nutritional quality, market value, and germination potential of stored produce (Hodges 1986; Borgemeister et al. 1998). Severe infestations frequently occur under poor storage conditions, particularly in traditional storage facilities used by smallholder farmers. Furthermore, the beetle is capable of attacking wooden structures, storage containers, and packaging materials, thereby enhancing its survival and facilitating its dispersal to new locations (Nansen et al. 2004).

Genetic variability within insect populations plays a significant role in determining their adaptability, ecological success, dispersal ability, host preference, and response to environmental pressures (Behura 2006). Consequently, studies of genetic diversity and population structure provide important information for understanding the ecology, invasion history, gene flow, and evolutionary dynamics of stored-product insect pests. Such knowledge also contributes to the development of effective surveillance programmes and sustainable pest management strategies (Fields and Phillips 2002). Molecular characterization has therefore become an essential approach for investigating insect biodiversity and monitoring the spread of economically important pest species. Although morphological examination remains the foundation of insect taxonomy, accurate identification can be difficult when specimens are damaged, immature, or exhibit morphological similarities with closely related species. Molecular techniques have therefore become indispensable complementary tools for species identification, taxonomic verification, and phylogenetic studies (Cruaud et al. 2017; Kjer 2004). Among the molecular markers employed in insect systematics, the nuclear 28S ribosomal RNA (28S rRNA) gene is particularly valuable because it contains conserved domains that facilitate primer design alongside variable regions that provide useful phylogenetic information. These characteristics make the 28S rRNA gene suitable for resolving taxonomic relationships and assessing evolutionary divergence across a wide range of insect taxa (Hillis and Dixon 1991; Whiting et al. 1997; Kjer 2004).

Phylogenetic analyses based on 28S rRNA gene sequences have been widely used to infer evolutionary relationships, clarify taxonomic placement, and evaluate genetic diversity among insect populations from different geographical regions. Such analyses provide valuable insights into species evolution, population connectivity, and historical dispersal patterns, thereby supporting the development of effective pest management and quarantine strategies (Whiting 2002; Kjer, 2004).

Despite the widespread occurrence and economic importance of *P. truncatus* in Nigeria, information on its molecular characterization and phylogenetic relationships remains limited. Most available studies have concentrated on the biology, ecology, distribution, and management of the pest, whereas relatively few have investigated its genetic diversity and evolutionary relationships. Molecular characterization of *P. truncatus* populations in Nigeria using the 28S rRNA gene will provide reliable baseline genetic information for species confirmation, facilitate comparisons with populations from other geographical regions, and improve understanding of the genetic structure of this invasive storage pest. Such information is essential for strengthening pest surveillance, improving quarantine programmes, and developing sustainable integrated pest management strategies.

Therefore, this study was undertaken to molecularly characterize *Prostephanus truncatus* populations collected from selected locations in Oyo State, southwestern Nigeria, using the nuclear 28S ribosomal RNA gene and to determine their phylogenetic relationships.

Materials and Methods

Study Area

The study was conducted in Oyo State, located in the South-western geopolitical zone of Nigeria. The state lies approximately between latitudes 7°03' and 9°12' N and longitudes 2°47' and 4°23' E, covering an estimated land area of about 28,454 km². Oyo State is bordered by the Republic of Benin to the west, Kwara State to the north, Osun State to the east, and Ogun State to the south (Oyo State Government 2023; National Bureau of Statistics 2022).

The climate of Oyo State is characterized by a tropical wet-and-dry climate with two distinct seasons: the rainy season, which usually extends from April to October, and the dry season, which spans from November to March. Mean annual temperatures generally range from 25 to 35°C, while annual rainfall varies between approximately 1,000 and 1,500 mm, depending on location within the state. Relative humidity is typically high during the rainy season and decreases during the dry months (NIMET 2023).

Oyo State encompasses three major vegetation belts, namely the rainforest in the southern region, the derived savanna in the central zone, and the Guinea savanna in the northern part of the state. These ecological zones support the cultivation of a wide range of food and cash crops, including maize (*Zea mays*), cassava (*Manihot esculenta*), yam (*Dioscorea* spp.), cowpea (*Vigna unguiculata*), and vegetables. Agriculture constitutes one of the major economic activities of the state's population, with crop production, livestock farming, and agricultural trading serving as important sources of livelihood. The favourable climatic conditions and extensive agricultural activities make Oyo State an important food-producing region and provide suitable conditions for the occurrence and proliferation of stored-product insect pests such as *Prostephanus truncatus* (National Bureau of Statistics 2022; Oyo State Government 2023).

Survey of the Occurrence of Prostephanus truncatus in Selected Markets in Oyo State

A field survey of *Prostephanus truncatus* from collected dried cassava chips was conducted in four (4) purposively selected markets in Oyo State, Nigeria The selected markets were Bodija Market (Ibadan), Sabo Market (Oyo Town), Oja-Agbe Market (Iseyin), and Sango Market (Saki) (Fig. 1).

The geographical coordinates and elevations of the sampling locations were recorded using a PG03 Mini Global Positioning System (GPS) receiver (Table 1).

Table 1
Geographical coordinates of market surveyed in Oyo State

Locations	Markets Surveyed	Geographical Coordinates
Ibadan	Bodija	7^{o} 26'. 18" N; 3^{o} 54'. 98" E
Oyo town	Sabo	7^{o} 52'. 67" N; 3^{o} 56'. 68" E
Iseyin	Oja-Agbe	7^{o} 58'. 18" N; 3^{o} 34'. 11" E
Saki	Sango	8^{o} 40'. 70" N; 3^{o} 23'. 60" E

All collected cassava chip samples were carefully labelled according to sampling location, date of collection, and store identification before being transported to the Entomology laboratory, Department of Zoology, University of Ibadan for insect identification, population assessment, and subsequent molecular analyses.

Morphological Identification of Prostephanus truncatus

Morphological identification of *Prostephanus truncatus* recovered from stored cassava chips was carried out using standard taxonomic keys and diagnostic morphological characteristics described for stored-product beetles (Haines 1991; Rees 2004). Adult specimens of *P. truncatus* were identified based on their distinctive morphological features, including a cylindrical, dark brown to blackish-brown body measuring approximately 3–4 mm in length, a strongly deflexed head that is largely concealed beneath the pronotum when viewed dorsally, well-developed longitudinal ridges on the elytra, and a characteristically truncate (squared) abdominal apex. Additional diagnostic features, including the structure of the pronotum and antennae, were also examined to distinguish *P. truncatus* from other bostrichid and stored-product beetle species encountered during the survey.

DNA Extraction

Genomic DNA was extracted from adult *Prostephanus truncatus* specimens following their morphological identification. A total of 28 adult individuals, comprising seven specimens from each of the four sampling locations (markets), were randomly selected for molecular analysis. Individual insects were aseptically transferred into sterile glass vials and processed separately to prevent cross-contamination.

Genomic DNA was isolated using the Quick-DNA™ Tissue/Insect Miniprep Kit (Zymo Research Corp., Irvine, CA, USA) according to the manufacturer's protocol, with minor modifications to optimize DNA yield and purity. The concentration and purity of the extracted DNA were determined using a spectrophotometer, while DNA integrity was evaluated by electrophoresis on a 1% agarose gel stained with an appropriate nucleic acid stain. Only high-quality DNA samples exhibiting acceptable purity and intact genomic bands were used for subsequent polymerase chain reaction (PCR) amplification.

PCR Amplification and Gel Electrophoresis

A partial fragment of the 28S ribosomal RNA (28S rRNA) gene was amplified using the primer pair Domain 2-3551F (5'-CGT GTT GCT TGA TAG TGC AGC-3') and Domain 2-4057R (5'-TCA AGA CGG GTC CTG AAA GT-3'), which generates an amplicon of approximately 680 bp (Gillespie et al. 2005). The primers were synthesized by Inqaba Biotec (West Africa Ltd.). The Domain 2 region of the 28S rRNA gene was selected because it provides valuable phylogenetic information for coleopteran insects and other insect taxa, owing to its combination of conserved and variable sequence regions that facilitate species discrimination and evolutionary inference (Sole and Scholtz 2010).

Polymerase chain reaction (PCR) was performed in a total reaction volume of 25 µL, comprising 12.5 µL of 2× PCR Master Mix, 4.0 µL of primer mix (2.0 µL each of forward and reverse primers), 2.0 µL of genomic DNA template, and 6.5 µL of nuclease-free water. Amplification was carried out using an ARKTIK thermal cycler under the following cycling conditions: initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 40 s, and extension at 72°C for 1 min; followed by a final extension at 72°C for 5 min. The amplified PCR products were resolved by electrophoresis on a 1.2% agarose gel prepared in 1× Tris–Acetate–EDTA (TAE) buffer and run at 100 V, 163 mA, and 16 W for 1 h. Following electrophoresis, the gel was stained with 8 mg mL^{-1} ethidium bromide, rinsed briefly in distilled water to remove excess stain, and visualized under a UV/white-light transilluminator (Spectroline Bio-Vision™). Images of the amplified DNA bands were subsequently captured and documented.

DNA Sequencing

A total of sixteen PCR amplicons, comprising four representative samples from each of the four markets, were selected for DNA sequencing. The purified PCR products were submitted to the Bioscience Centre, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, where bidirectional sequencing was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit according to the manufacturer's protocol.

Processing and Alignment of DNA Sequences

The obtained 28S rRNA gene sequences of *Prostephanus truncatus* were initially examined for quality using Chromas software. Low-quality bases and ambiguous nucleotide calls were manually edited to generate high-quality consensus sequences, which were subsequently assembled and edited using BioEdit version 7.2.5. The edited sequences were aligned using the ClustalW multiple sequence alignment algorithm implemented in MEGA version 7.0 to ensure accurate nucleotide alignment among all samples.

The aligned sequences were examined to identify nucleotide polymorphisms and assess genetic variation among the sampled populations. PCR amplification products were initially verified by agarose gel electrophoresis, and the resulting DNA bands were compared with a molecular weight marker (DNA ladder) to confirm the expected amplicon size for samples obtained from each sampling location.

Sequence Analysis

Phylogenetic relationships among the obtained sequences and closely related reference sequences retrieved from GenBank were inferred using the Maximum Parsimony (MP) method implemented in MEGA version 7.0. Prior to tree construction, all sequences were aligned using the ClustalW algorithm. Phylogenetic trees were generated using a heuristic search with the Tree Bisection–Reconnection (TBR)

branch-swapping algorithm. The robustness of the inferred phylogeny was evaluated by bootstrap analysis with 1,000 replicates. Bootstrap support values of 50% or greater were considered to represent well-supported clades.

Results

PCR Amplification of the Partial 28S rRNA Gene of Prostephanus truncate

A distinct 680 bp PCR amplification band was obtained from genomic DNA extracted from adult *Prostephanus truncatus* following amplification at an annealing temperature of 55°C, confirming successful amplification of the target 28S rRNA gene fragment. Figure 2. shows the PCR amplification of the partial 28S rRNA Domain 2 gene, yielding the expected 680 bp amplicon from genomic DNA extracted from adult *Prostephanus truncatus* collected from four markets in Oyo State. PCR amplification was successful in 26 of the 28 DNA samples analyzed. No amplification was observed for samples in lanes 2 and 20, corresponding to specimens collected from Bodija and Oja-Agbe markets, respectively.

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 M M 15 16 17 18 19 20 21 22 23 24 25 26 27 28 M

Bodija market (Ibadan) Sabo market (Oyo town) Oja-Agbe market (Iseyin) Sango market (Saki)

Figure 2. Agarose gel image of PCR products showing the amplification of the partial 28S rRNA gene (680 bp**)** from 28 *Prostephanus truncatus* specimens collected from four locations (Ibadan, Oyo Town, Iseyin, and Saki) in Oyo State, Southwestern Nigeria. Lanes 1–28 correspond to individual samples (seven per location), while M denotes the 100 bp DNA molecular weight marker.

Analysis of BLASTn Identification of Prostephanus truncatus Sequences

The BLASTn analysis of the partial 28S rRNA gene sequences confirmed that all DNA samples obtained from the four geographical locations (Ibadan, Oyo, Iseyin, and Saki) were identified as *Prostephanus truncatus*. Sequence identity with reference sequences deposited in the NCBI GenBank database ranged from **94% to 99%**, confirming the molecular identity of all analyzed specimens. Overall, higher bit scores (813–942 bits) were associated with higher sequence identities, reflecting stronger sequence similarity with the reference *P. truncatus* sequences (Table 2)

All samples produced an E-value of 0.0, indicating that the sequence matches were highly significant and that the probability of these alignments occurring by chance was essentially zero. This provides strong evidence for the accurate molecular identification of all specimens as *Prostephanus truncatus*. The successfully generated sequences were deposited in the NCBI GenBank database and assigned accession numbers MH647595–MH647609, thereby providing publicly accessible molecular records for *P. truncatus* populations collected from Ibadan, Oyo, Iseyin, and Saki in Oyo State, Nigeria (Table 2).

Table 2
Comparison of BLASTn Results of Partial 28S rRNA Gene Sequences of *Prostephanus truncatus* from Four Markets with GenBank Reference Sequences and Their Accession Numbers

Locations (Pests)	Blast N Identification	Identity (%)	Score (Bits)	Expect (E) Value	Accession Numbers
Ibadan 2	*P. truncatus*	98	905	0.0	MH647595
Ibadan 3	"	97	881	0.0	MH647596
Ibadan 4	"	97	878	0.0	MH647597
Oyo 1	"	97	898	0.0	MH647598
Oyo 2	"	96	881	0.0	MH647599
Oyo 3	"	98	937	0.0	MH647600
Oyo 4	"	98	924	0.0	MH647601
Iseyin 1	"	96	854	0.0	MH647602
Iseyin 2	"	94	813	0.0	MH647603
Iseyin 3	"	99	942	0.0	MH647604
Iseyin 4	"	99	939	0.0	MH647605
Saki 1	"	96	891	0.0	MH647606
Saki 2	"	95	837	0.0	MH647607
Saki 3	"	98	917	0.0	MH647608
Saki 4	"	97	883	0.0	MH647609

Phylogenetics Analysis of the Partial 28S rRNA Gene of Prostephanus truncatus

Maximum Parsimony (MP) analysis of the partial 28S rRNA gene dataset generated a consensus tree from two equally most parsimonious trees. The tree had a consistency index (CI) of 0.526316, a retention index (RI) of 0.389831, and a composite index (CI × RI) of **0.293045** for all sites, including parsimony-informative sites. A total of 528 nucleotide positions were included in the final alignment used for phylogenetic reconstruction.

The phylogenetic tree generated by the Maximum Parsimony method (Fig. 3) resolved the *Prostephanus truncatus* sequences into **three major clades (A, B, and C)** supported by bootstrap values of **50% or greater**. Clades A and B comprised *P. truncatus* specimens collected from all four sampling locations (Ibadan, Oyo, Iseyin, and Saki), whereas Clade C contained specimens from **Ibadan** and **Iseyin** only, irrespective of sample replicate. The phylogeny further showed that all analyzed specimens clustered within the *P. truncatus* lineage, confirming their genetic identity despite their separation into different clades. Several closely related sequence pairs were observed with **100% bootstrap support**, including

Saki 4 and Saki 2, Oyo 3 and Ibadan 3, and **Iseyin 2 and Oyo 2**, indicating a high degree of genetic similarity among these populations. In addition, **Iseyin 3, Saki 3**, and **Oyo 4** each formed distinct sister clades, reflecting their close evolutionary relationships.

The horizontal branch lengths of the phylogram represent genetic distances among the sampled populations. Specimens grouped within Clade C exhibited greater genetic divergence from those in Clades A and B, indicating a relatively higher level of sequence variation. Nevertheless, all *P. truncatus* specimens collected from the four markets in Oyo State formed a monophyletic group, demonstrating that they share a common evolutionary origin despite the observed genetic variation.

Discussion

The application of molecular biology techniques has become increasingly important in insect taxonomy, diagnostics, and phylogenetic studies because of their speed, sensitivity, accuracy, and reproducibility. In the present study, genomic DNA was successfully extracted from adult *Prostephanus truncatus* specimens using a modified extraction protocol without Proteinase K, demonstrating that high-quality DNA suitable for downstream molecular analyses can be obtained using this approach. Successful DNA extraction was further confirmed by the amplification of the targeted domain of the 28S ribosomal RNA (rRNA) gene, which produced an expected amplicon size of approximately 680 bp in the majority of the samples.

The 28S rRNA gene was selected because it contains both highly conserved and variable regions, making it an effective molecular marker for species identification and phylogenetic reconstruction in Coleopteran insects. Previous studies have successfully employed this marker in the phylogenetic analysis of *Tenebrio* species (Gillespie et al. 2004), members of the tribes Canthonini and Dichotomiini (Sole and Scholtz 2010), and *Callosobruchus maculatus* (Awa et al. 2011). Similarly, Sole and Scholtz (2010) reported that the 28S rRNA gene provides valuable phylogenetic information for resolving evolutionary relationships among beetles and other insect groups. More recently, Quellhorst et al. (2021) emphasized the increasing application of molecular markers in understanding the taxonomy, biology, and invasion pathways of stored-product beetles, including *P. truncatus*.

Among the 28 DNA samples analyzed, successful amplification was achieved in 26 specimens, while two samples from Bodija (Ibadan) and Oja-Agbe (Iseyin) failed to produce amplification products. The absence of amplification in these samples may have resulted from degraded DNA, insufficient template DNA, PCR inhibitors, or primer mismatch. Similar observations were reported by Ming et al. (2014), who noted that samples failing to amplify are generally considered unsuitable for molecular identification unless DNA quality can be improved. Nevertheless, the overall amplification success rate obtained in this study demonstrates the suitability of the extraction protocol and the robustness of the 28S rRNA marker for molecular characterization of *P. truncatus*. The high amplification efficiency may also be attributed to the multiple copies of ribosomal RNA genes within the insect genome, which increase the likelihood of successful PCR amplification.

Morphological identification remains an essential component of insect taxonomy; however, distinguishing closely related species, strains, or biotypes solely on morphological characteristics can be challenging because phenotypic traits may be influenced by environmental conditions (Fakrudin et al. 2006). Molecular markers therefore provide a more objective and reliable means of species identification. Sequence analysis of the amplified 28S rRNA fragments in the present study revealed no significant sequence variation among specimens collected from the different markets in Oyo State, confirming that all individuals belonged to the same species, *Prostephanus truncatus*. This finding demonstrates that the 28S rRNA gene possesses sufficient interspecific variation while remaining highly conserved within species, making it an effective marker for species delimitation and molecular identification. Similar conclusions have been reported by Holznagel et al. (2010), Cepeda et al. (2012), and Ming et al. (2014), who highlighted the usefulness of nuclear ribosomal genes in resolving species boundaries and phylogenetic relationships.

Phylogenetic analysis further supported the molecular identification of the sampled populations. All Nigerian isolates clustered within a single well-supported *P. truncatus* clade, confirming their common evolutionary origin and supporting their morphological classification. The Maximum Parsimony analysis generated two equally parsimonious trees with a consistency index (CI) of 0.526, retention index (RI) of 0.390, and composite index of 0.293. The moderate consistency index indicates that although some homoplasy exists within the dataset, the phylogenetic signal remains sufficiently informative for reconstructing evolutionary relationships. Likewise, the relatively low retention index suggests that certain nucleotide substitutions may have evolved independently, a phenomenon commonly observed in ribosomal DNA datasets because of the coexistence of conserved and variable regions.

The phylogenetic tree showed that isolates from Bodija (Ibadan), Sabo (Oyo town), Oja-Agbe (Iseyin), and Sango (Saki) markets formed a closely related cluster with previously published *P. truncatus* sequences, indicating minimal genetic divergence among populations within Oyo State. This limited genetic variation is likely a consequence of continuous movement of infested maize and dried cassava through commercial trading activities, resulting in extensive gene flow among populations. Frequent transportation and exchange of stored products between markets facilitate the dispersal of *P. truncatus*, thereby maintaining genetic homogeneity across geographical locations. Similar observations have been reported for African populations of *P. truncatus*, where recent invasion history and extensive commodity movement have resulted in relatively low genetic differentiation among populations (Quellhorst et al. 2021).

Conclusion

In this study, it was shown that the 28S ribosomal RNA (rRNA) gene can be used reliably to characterize *Prostephanus truncatus* population/groups as well as to perform phylogenetic studies with a high level of confidence in samples collected from Nigeria's Oyo State. All of the individuals in the samples studied were confirmed to be the same species based on the success of the amplification techniques and sequencing of this molecular marker (28S rRNA gene). The analysis of the genetic makeup of the

various populations as well as their pronounced similarity indicates that gene flow is occurring between the various populations most likely due to the translocation of contaminated agricultural products.

It is recommended that researchers conducting future studies include higher-repeating molecular markers (e.g., mitochondrial cytochrome oxidase I (COI), microsatellite, or single nucleotide polymorphisms (SNPs) across the genome), as these will give greater discrimination in assisting researchers to identify strain level differences and provide additional data to create a more complete picture of the population genetics, invasion routes, and dispersal behaviors of this economically critical pest, in addition to a greater range of geographical locations sampled.

Declarations

Financial interests:

The authors declare they have no financial interests.

Competing interests

The authors declare no competing interests.

Human Ethics and Consent to Participate declarations

Not applicable.

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Author Contribution

J.B and K.O.K.P conceived and designed the study, conducted the field research, and performed the morphological identification of the insect pest. T.K.I. collected and processed the insect specimens and analysed the data. C.A.L assisted with the molecular analyses and bioinformatics. All authors reviewed, edited, and approved the final manuscript.

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Figures

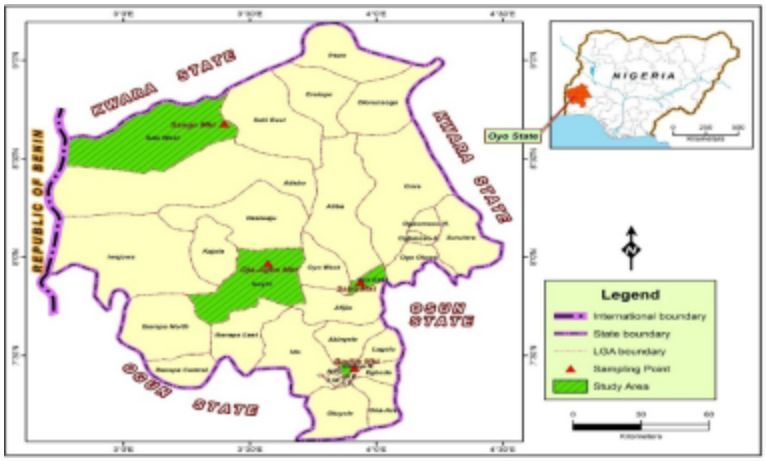


Figure 1

Map of Oyo State showing sampled markets

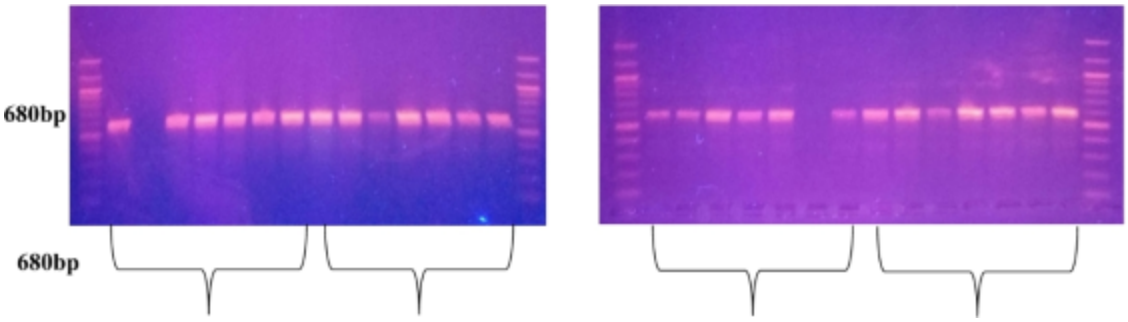


Figure 2

Agarose gel image of PCR products showing the amplification of the partial 28S rRNA gene (680 bp**)** from 28 *Prostephanus truncatus* specimens collected from four locations (Ibadan, Oyo Town, Iseyin, and Saki) in Oyo State, Southwestern Nigeria. Lanes 1–28 correspond to individual samples (seven per location), while M denotes the 100 bp DNA molecular weight marker.