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# Evaluation of DNA quality and molecular observation of Nile tilapia (*Oreochromis niloticus*) from Limpopo Province, South Africa

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**ABSTRACT**

**In this study, the ability of the Quick-DNA™ Tissue/Insect Miniprep Kit and Chelex® methods to extract DNA from *O. niloticus* skin, muscle, and gill tissue was compared. The quantity and purity of the DNA were measured with a NanoDrop spectrophotometer. Based on the results obtained, it appears that the DNA extracted using the Kit has good quality based on A260/280 (1.67–1.98), and the Chelex method (1.52-1.81) was acceptable. ANOVA for the amount of nucleic acid revealed a significant difference between muscle and skin with gill tissue ($P < 0.05$). However, the skin of O. niloticus subjected to both methods was the best at extracting DNA (1.89-1.81). The extracted DNA was also studied by 28S ribosomal DNA and COI of mitochondrial DNA genes. Phylogenetic analysis based on 28S rDNA and COI of mtDNA placed the South African population of *O. niloticus* in a clade with other related species with a posterior probability value of 1.00. Finally, the molecular results showed that 28S ribosomal DNA is a suitable marker for the identification of *O. niloticus*. In conclusion, precise identification of *O. niloticus* is critical for breeding for farmers and commercial sectors.**

## Introduction

Recently, aquatic research has focused on the molecular level, such as AFLPs, SNPs, microsatellites, and sequencing, which require reliable, inexpensive, and quick protocols for extracting DNA (Montero-Pau *et al*., 2008). Several DNA extraction methods have been developed for obtaining genomic DNA. However, the most critical aspects of DNA extraction methods are safety, availability, reliability, low cost, and speed. Nevertheless, some protocols, such as chloroform-based methods for extracting DNA, harm humans (Aminisarteshnizi 2022). On the other hand, DNA extraction is costly in terms of money and time; therefore, molecular studies are limited. Many researchers have highlighted the cost-reducing potential of Chelex-based DNA extraction methods, which use chelating ion exchange resins (Shokoohi and Eisenback 2023). After cell lysis, Chelex can attach to polar cellular components. All nonpolar nuclear RNA and DNA will stay above the Chelex in the solution. Chelex can prevent the reduction of DNA quality by chelating metal ions (Casquet *et al.,* 2012). The most widely cultivated fish in aquaculture worldwide is *O. niloticus*. Nile tilapia grow quickly and are resistant to stress and disease. This fish can thrive in various environmental conditions (Moyo and Rapatsa 2021). Nile tilapia feed at low trophic levels (Hlophe and Moyo 2011), and they reproduce in captivity and short generation times (Hlophe and Moyo 2014). Studies on this fish should provide more detailed information at the molecular level. Therefore, this study aimed 1) to assess the quality of DNA from *O. niloticus* using various methods and 2) to study the phylogenetic position of the studied tilapia in Limpopo Province

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using 28S rDNA and COI sequences of mtDNA genes.

## Material and Methods

*O. niloticus* specimens were collected from the Aquaculture Research Unit at the University of Limpopo, located at 23.89091° S, 29.74037° E (Fig. 1), in 2021.

**DNA extraction**

**Chelex method**

The Chelex method was used to extract DNA (gDNA) (Yue and Orban 2001). The supernatant was then stored at -20°C.

**Quick-DNA™ Tissue/Insect Miniprep Kit**

DNA was extracted using a kit following the manufacturer's protocol (Zymoresearch, USA; https://zymoresearch.eu/collections/quick-dna-tissue-insect-kits). The supernatant was then stored at -20°C.

**Quality and quantity of DNA**

To estimate the amount of extracted DNA, a Thermo Scientific NanoDrop™ One Spectrophotometer from Germany was used. One microliter of each sample was measured, and the process was repeated three times. The purity of each sample was checked by determining the A260/280 ratio.

**Statistical methods**

To analyze the effects of different extraction methods on different parts of tilapia fish, ANOVA was performed using SPSS Statistical Software System 11.01 from SPSS Chicago, IL, USA. A pairwise comparison based on the Tukey method at the 5% level was used to identify significant differences in mean values. Additionally, principal component analysis (PCA) was conducted using XLSTAT, a two-way table of different parts of tilapia fish and extraction methods. The variables were weighted with the inverse of the standard deviation for the different scales of the variables, as recommended by Addinsoft (2007).

**PCR amplification and phylogenetic analysis**

The extracted DNA, as explained above, was used for PCR amplification of the 28S rDNA region, the D2A and D3B primers (Shokoohi *et al*., 2023) were used, and for the COI gene fragments of mtDNA, the primers FishF1 and FishR1 (Soliman *et al*., 2017) were used. PCR was carried out with a DNA template, PCR Master Mix Red, primers, and $ddH_2O$.



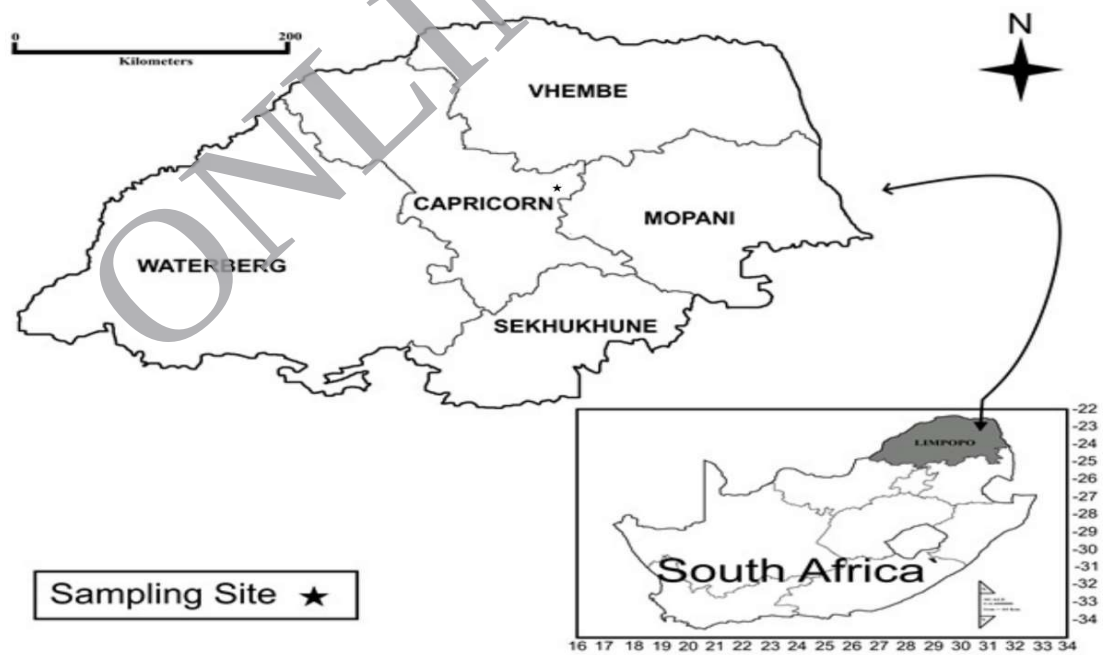


**Figure 1: Location of the study area**

The amplification process was conducted with an Eppendorf master cycler gradient following a specific program. After amplification, we evaluated the DNA bands by loading the product on a 1% agarose gel in TBE buffer and staining the bands with Safe-view Classic. Finally, Inqaba Biotech purified the PCR products for sequencing. Additionally, as outgroups, *Clarias gariepinus* (AF323692) was selected for 28S rDNA and COI of the mtDNA trees. The sequences were analyzed and edited using BioEdit (Hall 1999) and aligned using CLUSTAL W (Thompson *et al*., 1994). The Bayesian inference method was used to generate phylogenetic trees with the MrBayes 3.1.2 program (Ronquist and Huelsenbeck 2003). The GTR+I+G model was selected for the 28S rDNA and COI of the mtDNA trees using jModeltest 2.1.10 (Darriba *et al*., 2012). The selected model was then initiated with a random starting tree and run with the Markov chain Monte Carlo (MCMC) method for $10^6$ generations. The new partial 28S rDNA (OR230127) and COI of mtDNA (OR230246) were deposited in GenBank.

## Results and Discussion

### DNA quality

A NanoDrop instrument was used to verify the quality and quantity of the extracted DNA samples. To successfully amplify PCR products, 20–200 ng/µL nucleic acid is needed. All samples had nucleic acid concentrations greater than 20 ng/µL, making them suitable for PCR amplification. The ANOVA results for the amount of nucleic acid showed a significant difference between muscle and skin with gill tissue ($P < 0.05$) for both methods. In this study, the 260/280 ratio ranged from 1.52–1.98. The kit method produced DNA samples with purity ratios of 260/280 between 1.67–1.98, while the purity ratio of samples extracted by the Chelex method was between 1.52–1.81 (Table 1). Both methods produced the best DNA quality for the skin part (1.89-1.81). A sample purity ratio >1.9 indicated the presence of proteins. However, more than 1.9% of the gill tissues were affected by the Kit method. In both methods, samples from the muscle had salts (A260/A280 ratios of <1.7). The total protein content differed significantly between the kit and Chelex methods ($P<0.05$) (Figure 2). The kit method produced more total protein than did the Chelex method. Total protein in the Kit from gill, skin, and muscle differed significantly ($P<0.05$). Total protein from gill, skin, and muscle in the Chelex method did not differ significantly ($P>0.05$). PCA revealed that a set of variables explained 96.11% of the variability in the first two axes (Fig. 3). The first principal component (F1) explained 70.21% of the total variance. F1 was positively correlated with DNA for gill tissue extracted with the kit method and negatively correlated with DNA for muscle tissue according to both methods. The second principal component, F2, explained 25.90% of the variance. F2 was positively correlated with the amount of gill DNA extracted via the Chelex method and negatively correlated with the amount of skin DNA extracted via both methods (Fig. 3). PCA clearly revealed that DNA extracted from different parts of the tilapia fish was separated by both methods.

**Table 1: Mean purity and quantity of genomic DNA extracted from different parts of tilapia fish by the Kit and Chelex method (mean±SE) (n= 4 in each case)**

| Sample | Nucleic acid (ng/uL) | 260/280 | 260/230 | A260 | A280 |
|---|---|---|---|---|---|
| Muscle (Kit) | $37.8^{a}$±1 | 1.67±0.01 | 0.88±0.01 | 0.155±0.01 | 0.102±0.01 |
| Muscle (Chelex) | $32.8^{a}$ ±2 | 1.52±0.01 | 0.245±0.01 | 0.256±0.01 | 0.153±0.01 |
| Skin (Kit) | $35.2^{a}$ ±2 | 1.89±0.01 | 1.079±0.01 | 0.305±0.01 | 0.154±0.01 |
| Skin (Chelex) | $32.6^{a}$ ±2 | 1.81±0.01 | 0.65±0.01 | 0.252±0.01 | 0.139±0.01 |
| Gill (Kit) | $57.46^{b}$±3 | 1.98±0.01 | 1.33±0.01 | 0.749±0.01 | 0.389±0.01 |
| Gill (Chelex) | $53.6^{b}$ ±4 | 1.59±0.01 | 6.23±0.01 | 0.272±0.01 | 0.171±0.01 |

**Means within the same column with different superscripts are significantly different ($P < 0.05$).**

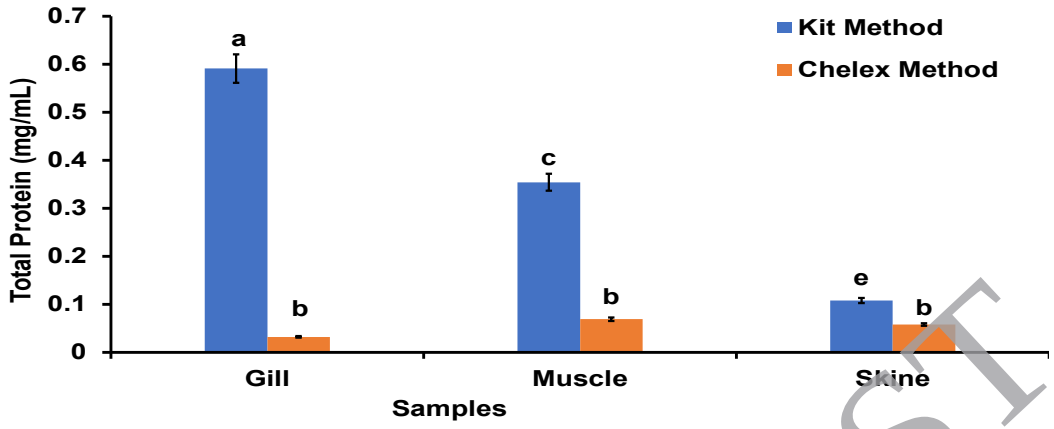


**Figure 2: Total protein was obtained for all samples extracted using the Chelex and Kit methods**

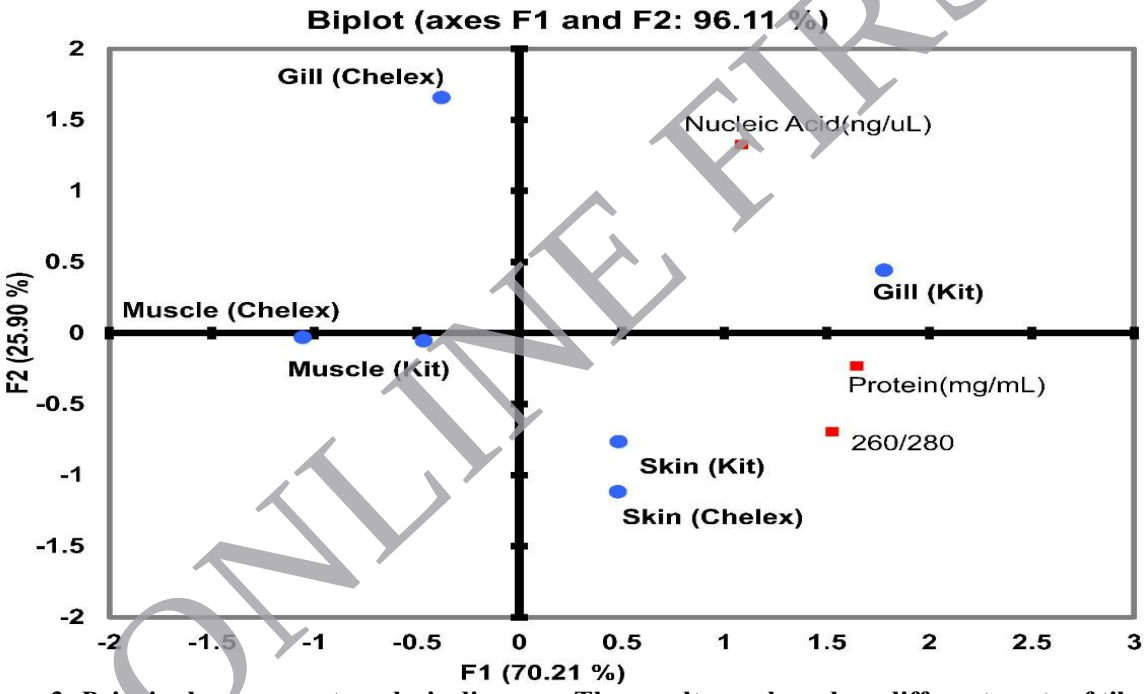


**Figure 3: Principal component analysis diagram. The results are based on different parts of tilapia fish, and DNA was extracted in two different ways**



**Molecular characteristics and phylogenetic relationships of *O. niloticus***

Phylogenetic analysis of the 28S rDNA (Fig. 4) and COI (Fig. 5) trees revealed that the studied population of *O. niloticus* was in the same clade as other populations of *O. niloticus,* with posterior probability values of 1.00. The 28S rDNA of the tested population of *O. niloticus* from South Africa showed 99% similarity with that of a population of the same species from the USA (XR003216136). Furthermore, our mtDNA COI of *O. niloticus* sampled from South Africa was 99% similar to that of *O. niloticus* from Thailand (MG438454). Additionally, the studied population of *O. niloticus* showed 99% similarity with a population of the same species from India (OR143704). Population genetic studies significantly depend on polymerase chain reaction (PCR). The basic material for this type of

study is high-quality DNA. In this study, two DNA extraction methods for *O. niloticus* were compared. The results showed that both methods produced similar quality results. The Chelex method is simple, rapid, and not harmful to human health (Casquet *et al*., 2012). The results agree with those of several studies. Ardura *et al*. (2010) showed that the Chelex method performs well on ethanol-stored and fresh Amazonian commercial fish samples. Casquet *et al.* (2012) demonstrated that the Chelex method is a suitable and efficient DNA extraction procedure for large-scale barcoding projects. Lucentini *et al*. (2006) reported that a modified protocol for Chelex was suitable for DNA extraction from fins and scales. The results revealed that the Chelex method produced high-quality DNA, which can be used for further analysis. A multivariate statistical method called principal component analysis (PCA) was used to determine the most significant variables in the data. The complexity of the data resulted from different extraction methods and fish parts. PCA revealed that skin tissue was the discriminating parameter. The PCA values indicated a significant difference between the extraction methods and matrices when all variables were considered. Based on these results, the Chelex method is recommended for the extraction of DNA from *O. niloticus* skin tissue.




**Figure 4: Phylogenetic tree based on 28S rDNA sequences, including the South African population of *O. niloticus* (bold)**


**Figure 5: Phylogenetic tree based on the COI sequences of mtDNA, including the South African population of *O. niloticus* (bold)**



In this study, DNA was extracted from three different parts of *O. niloticus* to determine the most suitable part for PCR amplification. The results showed that extracting DNA from the skin using both methods produced high-quality DNA, which was suitable for PCR amplification. Furthermore, the results showed that the total protein in the skin tissue was less than that in the gill and muscle tissue. Besbes *et al*. (2011) demonstrated that the greatest amplicon length of DNA was obtained from fresh samples of sardine and anchovy muscle tissues, but the amount of total protein was high and needed purification. Khoirunnisa *et al*. (2018) studied DNA extraction from fish fillets using the Chelex method. They reported a high amount of total protein. The quality of DNA measured by Nanodrop can be significantly impacted by different components present in the sample matrices, such as polyphenols, lipids, and polysaccharides. The specific procedures used to remove contaminating molecules could account for the variations observed in the protein amount between the Kit and Chelex methods. In this study, for the Chelex method, protein or salt was used because the extracted DNA was not purified. However, during PCR amplification, all the electrophoretic bands were relatively clear. Several

phylogenetic studies of Nile tilapia have shown that rDNA and mtDNA are useful for tilapia identification (Perina *et al*., 2011). Studies on DNA as a marker for animal taxonomy opened the door to improving the knowledge of the phylogenetic position of the animals (Martins *et al*., 2004). Chromosomal mapping of 45S rDNA genes has been used to answer phylogeographic questions in many fish species (Martins *et al*., 2004). Dunz and Schliewen (2013) studied the molecular phylogeny of various tilapia species and reported that *O. niloticus* is well distinguished from other species. The current study yielded the same outcome. Similarly, Ekerette *et al*. (2018) used mtDNA to study the phylogeny of *O. niloticus* in Nigeria. Their results showed that *O. niloticus* stands separately from other species of *Orechromis*. However, the relationships between *O. niloticus* and other species, such as *O. aureus* and *O. mosambicus,* should be investigated.

## Conclusion

In this study, two methods for extracting DNA from the skin, muscle, and gill of *O. niloticus* were explored. Both methods were successful in extracting DNA from all samples. Therefore, the Chelex method is a suitable and efficient DNA extraction procedure for barcoding projects. Surprisingly, the skin was found to be the best source of DNA, despite most studies using muscle tissue for DNA extraction. The extracted DNA was then analyzed using 28S rDNA and COI mtDNA genes. Based on these markers, the results of the phylogenetic analysis placed the South African population of *O. niloticus* in a clade with other closely related species, with a posterior probability value of 1.00. Finally, it was concluded that 28S rDNA is a suitable marker for identifying *O. niloticus*. Precisely identifying *O. niloticus* is crucial for breeding in both the farming and commercial sectors.

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## Conflict of interest

The authors declare that they have no conflicts of interest.

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