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Morphological and Molecular Characterization of Duckweed (Lemnaceae*)* in Selected Wetlands and Pond Waters of Tharaka-Nithi County, Kenya

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Abstract

Duckweeds are aquatic herbs adapted to various geographic and climatic zones, with significant applications in wastewater treatment, animal feeds, biofuel production, and as a culinary ingredient in some Asian countries. In Kenya, including Tharaka-Nithi County, duckweed has attracted the attention of farmers as a feed supplement for livestock. However, commercial extraction poses a threat to the survival of the plant and its ecological roles, necessitating its conservation and the promotion of sustainable utilization. This study aimed at characterizing local duckweed species to understand their taxonomic variation and distribution in Tharaka-Nithi County. One hundred and forty-four samples were collected from selected wetlands and ponds, with GPS coordinates and elevation of sampling points recorded for analysis of their distribution. Morphological features of duckweed were studied, and the DNA was extracted for molecular characterization using DNA barcoding markers. Based on morphological characterization, samples were grouped into nine clones. All nine clones had parallel veins and obovate fronds with rounded apices. Frond symmetry, color, border, and length varied among clones. Morphological data suggested that the clones belonged to the same genus, consistent with previous studies. Further, molecular characterization that was done using RBCL and matK genes successfully confirmed that they were duckweed species. Six RBCL gene amplification products were sequenced, with BLAST search results indicating the genus *Lemna*. Three samples collected from Chogoria, Gatithini, and Ikumbo were identified as *Lemna minor*. In contrast, samples collected from Marimanti, Kathwana, and Kaanwa were identified as *Lemna turionifera*, *Lemna aequinoctialis*, and *Lemma perpusilla,* respectively. *Lemna minor* and *Lemna turionifera* were predominant in treated sewage ponds, while *Lemna aequinoctialis* and *Lemma perpusilla* were found on still waters in swamps and fishponds, respectively. This study provides baseline information that can be used in formulating conservation and utilization policies for duckweed in Tharaka-Nithi County and beyond.

Keywords: *Duckweed, characterization, taxonomic variation, conservation, sustainable utilization*

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1. Introduction

Aquatic plants play a vital role in maintaining the balance and sustainability of aquatic ecosystems. They form essential components of the food web, generate dissolved oxygen, and provide shelter for insects, invertebrates, amphibians, and small fish (Irfan et al., 2019). Among these, duckweeds—tiny floating plants from the family *Lemnaceae*—stand out due to their rapid growth, high nutrient content, and ease of harvesting (Baek et al., 2021). These attributes make duckweed an excellent candidate for various industrial and environmental applications such as wastewater treatment, animal feed, human food, and renewable energy production (Pagliuso et al., 2022). Environmentally, duckweed can absorb heavy metals and pollutants from water and has been utilized in producing biofuels like biogas, ethanol, and synthetic fuels such as diesel and kerosene (Ziegler et al., 2015; Cheng & Stomp, 2009; Baliban et al., 2013). However, increasing industrial demand raises concerns about overexploitation, which could endanger natural populations and disrupt ecological functions.

Accurate taxonomic classification and understanding of local duckweed biodiversity are crucial for preventing overuse and ensuring conservation (Ziegler et al., 2023). The International Union for Conservation of Nature (IUCN) emphasizes biodiversity preservation at the genetic, species, and ecosystem levels (Ali et al., 2015). Reliable species identification requires the integration of morphological and molecular data (Hau & Mah, 2024). Advances in sequencing and bioinformatics have fueled growing scientific interest in duckweed, leading to the successful sequencing of five species from *Spirodela* and *Lemna*. So far, 38 species have been identified, with molecular taxonomy recognized as the most reliable approach for classification (Al-Dakhil et al., 2021).

Molecular taxonomy utilizes advanced tools such as PCR, qPCR, DNA barcoding, and high-throughput sequencing, offering greater accuracy than earlier chemotaxonomic techniques (Al-Dakhil et al., 2021). Due to the minute and simple morphology of duckweed, visual identification is difficult and often requires specialists. Traditional approaches like flavonoid profiling and allozyme analysis once provided preliminary differentiation (Bog et al., 2019), but DNA-based taxonomy now delivers more precise genetic insights. The Consortium for the Barcode of Life (CBOL) proposed seven plant barcode markers—four plastid coding genes (*rpoB, rpoC1, rbcL, matK*) and three non-coding spacers (*atpF-atpH, psbK-psbI, trnH-psbA*). For duckweed, combinations such as *matK*, *rbcL* + *trnH-psbA*, or multilocus systems improve classification accuracy (Zhang & Azizullah, 2020).

Despite their ecological and industrial value, many duckweed species remain unclassified in regions like Tharaka-Nithi County, where wetlands, ponds, and wastewater sites are abundant. The lack of precise taxonomic data hampers conservation efforts and sustainable application. Given that duckweed species often appear morphologically identical, molecular characterization using plastid coding genes (*rbcL*, *matK*) and non-coding spacers (*psbK-psbI*, *trnH-psbA*) provides the most reliable identification results.

1.1 Problem Statement

The ecological importance of duckweeds is increasingly at risk due to ongoing climate change and expanding industrial uses such as wastewater treatment, livestock feed, bioenergy production, and inclusion in human diets. Growing awareness of their economic potential has encouraged large-scale harvesting, which, if poorly managed, may threaten natural populations and even cause local or global extinction of some species. Such losses would disrupt the

essential ecological roles duckweeds perform—providing food for aquatic organisms, releasing oxygen, offering shelter, and absorbing excess nutrients. In Tharaka-Nithi County, Kenya, limited taxonomic knowledge and poor documentation hinder effective conservation and sustainable management. Without accurate species identification, conservation planning and industrial utilization become inefficient. This study addresses the gap by identifying and classifying local duckweed species using morphological and molecular methods. The results provide vital data for sustainable use, biodiversity conservation, and informed policy development to ensure the long-term ecological and economic stability of duckweeds.

1.2 Research Objective

To characterize duckweed species using morphological and molecular methods for their variation in taxonomic characters and their distribution in the selected wetlands and pond waters in Tharaka-Nithi County.

2. Materials and Methods

2.1 Morphological Characterization of Duckweed Samples

Duckweed samples were obtained from nine locations across Tharaka-Nithi County, including Chogoria, Magutuni, Ikumbo, Chuka, Kaanwa, Kathwana, Marimanti, Mukothima, and Gatithini. Each pond's surface was divided into four quadrants by drawing imaginary lines across the water, and four fronds were collected from different plants in each section. Details such as habitat type, collection number, GPS coordinates, site elevation, and both habitat and species characteristics were documented to assess duckweed distribution in the area. In total, 144 samples were placed in clearly labeled microcentrifuge tubes and transported to Chuka University for analysis. Fresh specimens were examined to observe fronds, roots, and other features following the morphological guidelines of Ceschin et al. (2016), which consider fourteen qualitative and quantitative traits. Using a magnifying lens, frond shapes were compared to botanical standards, and color, symmetry, venation, and apex form were described. Measurements of length, width, and root attachment points were recorded to calculate frond area and length-to-width ratios. The number of fronds per cluster and visible veins was also counted. Observations of aerenchyma distribution were made to distinguish internal air space patterns. Once all morphological assessments were completed, the samples were frozen at −80 °C for subsequent DNA extraction and molecular analysis.

2.2 Molecular characterization

2.2.1 DNA Extraction

Total DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, United States) following the manufacturer's instructions. The fresh tissue DNA quality and quantity were determined by UV absorbance at 260 nm wavelength using the Nanodrop 2000C (Thermo Fisher Scientific, Inc., Waltham, MA, United States). DNA was stored at -20 ℃ until use.

2.2.2 Primer selection and PCR assay

DNA barcoding markers were selected based on reference duckweed sequences recommended by the Plant Working Group of the Consortium for the Barcode of Life (CBOL) (Kress et al., 2007; Hollingsworth et al., 2009). Two plastid coding genes, matK and rbcL, were utilized in this study. Primer sequences were synthesized by the Bioscience Eastern and Central Africa Hub (BecA), diluted with ultrapure water to 100 pmol for master stocks, and further diluted to

10 pmol working stocks stored at −20 °C. Each PCR reaction contained 50 ng genomic DNA, 5 pmol of each primer, and 2× PCR Master Mix (Promega, USA). Amplification involved denaturation at 94 °C, annealing at 48 °C (matK) or 50 °C (rbcL), and extension at 72 °C. Purified PCR products followed Hao and Stephen's (2008) methods and were size-separated on agarose gels before direct sequencing.

2.3 Determination of the distribution of Duckweeds in Tharaka Nithi County

The habitats where duckweed samples were collected included well-established treated sewage ponds, wetlands with swampy, water-logged soils, and fishponds. The GPS coordinates and habitat characteristics were recorded for each study site. Altitudes of the study sites were also taken. The morphological data and DNA barcoding results were correlated with the field data to analyze the distribution of duckweed species across Tharaka-Nithi County.

2.4 Data Analysis

Morphological data were analyzed using RStudio, while molecular sequence data were processed in MEGA11. Quantitative traits such as frond length, width, number of connected fronds, frond area, length-to-width ratio, distance from frond base to root attachment, and number of veins were subjected to Analysis of Variance (ANOVA) in RStudio to assess significant variations among samples from fishponds, wetlands, and wastewater ponds. A Principal Component Analysis (PCA) was also performed to determine the contribution of each variable in distinguishing duckweed species, with results visualized using PCA biplots.

Purified PCR products were sequenced at the Bioscience Eastern and Central Africa Hub (BecA), ILRI, Nairobi, using an ABI3730 automated sequencer with the same matK and rbcL markers. Low-quality and ambiguous nucleotide bases were removed, and multiple sequence alignments were created using Clustal W. The aligned sequences were analyzed in MEGA11 under specific parameters (IUB matrix, transition weight 0.50, and 30% cutoff). Processed sequences were converted to FASTA format via Chromas, trimmed, and subjected to BLAST searches for species identification based on lowest E-values and highest identity scores. A phylogenetic tree illustrating evolutionary relationships was then constructed in MEGA11 using the Maximum Likelihood method with the Kimura 2-parameter model.

3. Results and Discussion

3.1 Results

3.1.1 Morphological Characterization of Duckweed Plants

A total of 144 duckweed samples were collected from the field, with 16 samples obtained from each study site. Specimens from individual sites displayed similar morphological features and were therefore classified as clones, leading to the identification of nine distinct duckweed clones. According to the qualitative morphological traits summarized in Table 3.1, all clones possessed obovate fronds with parallel venation and rounded apices. However, noticeable differences occurred in frond symmetry, coloration, hyaline presence, and aerenchyma extension. Clones from Chogoria, Chuka, Marimanti, Kathwana, Mukothima, and Magutuni had asymmetric fronds, while those from Gatithini, Ikumbo, and Kaanwa were symmetric, implying possible genetic or environmental influences. Hyaline tissue, essential for buoyancy and moisture retention, was absent in clones from Chogoria, Kaanwa, and Magutuni but present in others, suggesting structural variation linked to water conditions. Frond coloration also varied—dark green fronds were recorded in Chogoria, Gatithini, Kaanwa, and Magutuni,

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whereas Chuka, Marimanti, Ikumbo, Kathwana, and Mukothima exhibited lighter shades, possibly reflecting differences in chlorophyll concentration or light exposure. Aerenchyma extension, important for flotation, was limited in Gatithini and Magutuni clones but extended in the remaining populations, likely enhancing their buoyant capacity.

Table 1: Qualitative morphological Description of Duckweed

LOCATION	FSH	FS	FB	FC	FA	A	V
1. Chogoria	Obovate	Asymmetry	no hyaline	dark green	Rounded	Extended	Parallel
2. Gatithini	Obovate	Symmetry	Hyaline	dark green	Rounded	not extended	Parallel
3. Chuka	Obovate	Asymmetry	Hyaline	light green	Rounded	Extended	Parallel
4. Marimanti	Obovate	Asymmetry	Hyaline	light green	Rounded	Extended	Parallel
5. Ikumbo	Obovate	Symmetry	Hyaline	light green	Rounded	Extended	Parallel
6. Kathwana	Obovate	Asymmetry	Hyaline	light green	Rounded	Extended	Parallel
7. Kaanwa	Obovate	Symmetry	no hyaline	dark green	Rounded	Extended	Parallel
8. Mukothima	Obovate	Asymmetry	Hyaline	light green	Rounded	Extended	Parallel
9. Magutuni	Obovate	Asymmetry	no hyaline	dark green	Rounded	not extended	Parallel

Key: FSH – frond shape; FS – frond symmetry; FB – frond border; FC – frond colour; FA– frond apex; A– aerenchyma; V- Venation.

The variations observed highlight the morphological diversity of duckweed across different habitats within the region.

According to the quantitative morphological traits summarized in Table 2, ANOVA results for frond length (FL) and width (FW) revealed highly significant differences among clones ($p < 0.0001$), indicating notable morphological variation across sites. Frond length ranged from 5.2 mm in Gatithini to 5.84 mm in Ikumbo, while frond width varied from 2.27 mm in Chogoria to 5.40 mm in Kaanwa. The frond length-to-width ratio differed distinctly, with Chuka clones showing the highest ratio (2.74±0.17) and Gatithini the lowest (0.62±0.10). Vein number varied among clones, though ANOVA showed no significant differences, suggesting that certain morphological traits remain relatively conserved despite environmental variation.

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Table 2: Quantitative Morphological Description of Duckweed.

LOCATION	FL	FW	NCF	FA	FL/FW	DISTANCE	NO OF VEINS
1. Chogoria	5.6±0.33 b	2.27±0.21 e	2.67±0.47 a	13.8±0.66 b	2.03±0.87 ab	0.17±0.05 b	1.33±0.47
2. Gatithini	5.2±0.36 d	4.07±0.21 bd	3.67±0.47 a	13.8±0.66 b	0.62±0.10 c	0.37±0.05 ab	2.67±0.46 ab
3. Chuka	5.8±0.36 a	2.53±0.21 e	4.33±0.45 a	13.8±0.66 b	2.74±0.17 a	0.33±0.05 ab	1.33±0.48
4. Marimanti	5.8±0.32 cd	3.53±0.0.49 d	3.67±0.44 a	13.8±0.66 b	0.97±0.15 bc	0.40±0.08 ab	3.00±0.82 ab
5. Ikumbo	5.84±0.37 ab	5.33±0.17 a	3.33±0.47 a	13.8±0.66 b	1.13±0.04 bc	0.53±0.09 a	3.67±0.47 a
6. Kathwana	5.8±0.31 ab	4.60±0.08 abc	3.00±0.82 a	13.8±0.66 b	1.34±0.03 bc	0.40±0.08 ab	3.00±0.82 ab
7. Kaanwa	5.7±0.32 c	5.40±0.08 a	4.00±0.83 a	13.8±0.66 b	0.72±0.01 c	0.17±0.05 b	1.33±0.47
8. Mukothima	5.4±0.36 a	4.93±0.39 ab	4.33±0.94 a	13.8±0.66 b	1.41±0.13 bc	0.40±0.08 ab	4.00±0.82 a
9. Magutuni	5.8±0.36 cd	3.67±0.33 cd	3.67±0.47 a	13.8±0.66 b	0.95±0.03 c	0.43±0.12 ab	4.00±0.82 a

Key: FL – frond length; FW – frond width; NCF – number of contiguous fronds; FA – frond area; FL/FW – frond length/frond width; Distance – Distance between frond-base and root-attachment.

The variations observed highlight the morphological diversity of duckweed across different habitats within the region. Means followed by the same letter in a column are not significantly different based on Tukey's multiple comparisons test, $p< 0.05$.

Principal Component Analysis (PCA) was conducted to interpret correlations among quantitative and qualitative morphological traits of duckweed clones (Figure 1). PC1 explained the greatest variance (32.2%), with frond color and area showing positive correlations, while frond width, base-to-root distance, number of contiguous fronds, and frond border correlated negatively. PC2 differentiated clones mainly by frond elongation and symmetry, with frond length and length-to-width ratio positively correlated, and frond symmetry, shape, and aerenchyma negatively correlated. Clones from Chogoria and Chuka showed distinct morphological traits with high PC1 values, while Mukothima and Marimanti had negative loadings, indicating smaller, less symmetrical fronds.

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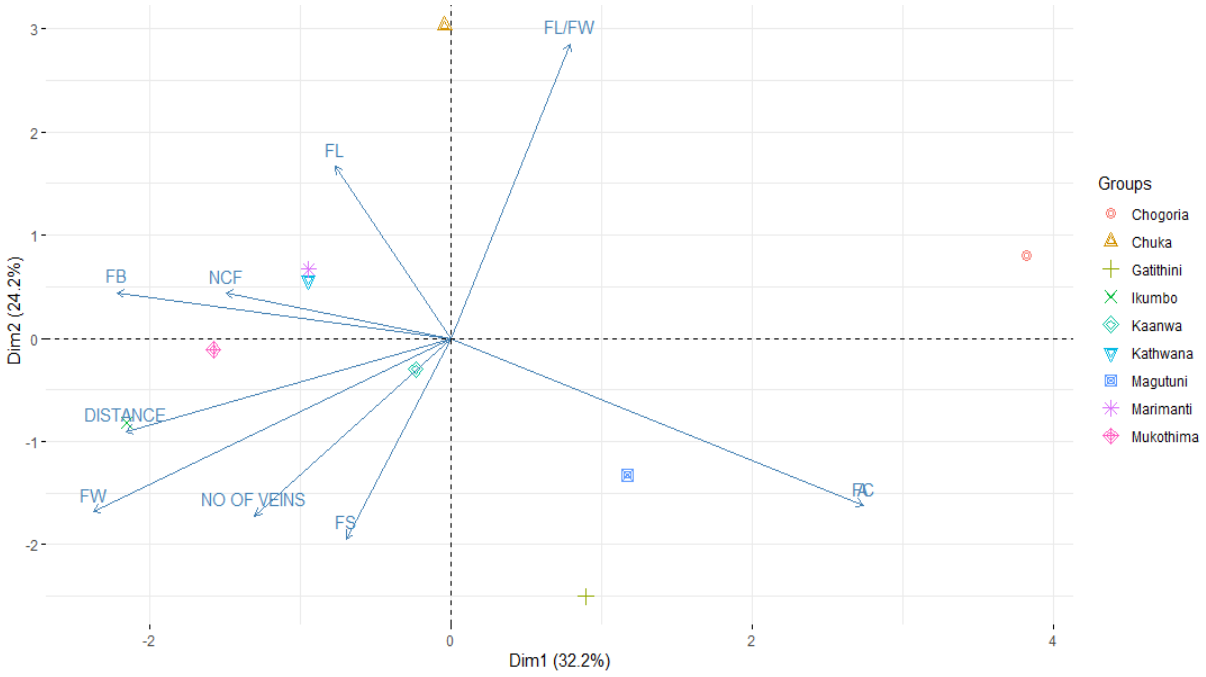

Figure 1: Two-dimensional biplot of duckweed clones on principal component axes according to qualitative and rescaled quantitative data traits.

Principal component 1 represented by 32.2% of the total variance, and principal component 2 represented by 24.2% of the total variance. FSH – Frond shape; FS – frond symmetry; FB – frond border; FC – frond colour; FA – frond apex; A Arenchyma, Frond length; FW – frond width; NCF – number of contiguous fronds; FA – frond area; FL/FW – frond length/frond width; Distance – Distance between frond-base and root-attachment.

3.1.2 PCR Assay

Samples amplified by the matK primer yielded medium-sized bands (Figure 3.2A). Samples amplified by the rbcL primer yielded longer bands (Figure 3.2B). Six PCR products: 1 – Chogoria; 2 – Gatithini; 4 – Marimanti; 5 – Ikumbo; 6 – Kathwana; 7 – Kaanwa passed QC hence successfully sequenced. PCR products from 3-Chuka, 8-Mukothima, and 9-Magutuni failed the QC test.

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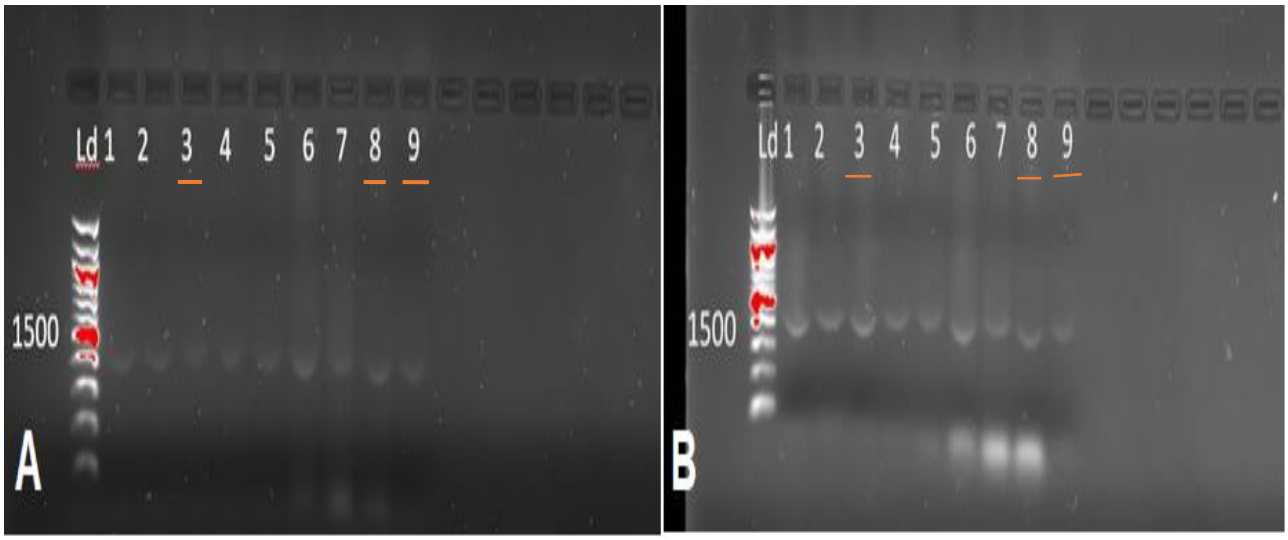

Figure 2: The image shows amplification of matK (A) and rbcL (B) genes using a 1.5kb ladder (Ld).

On gel A and B, Ld represents ladder, 1 – Chogoria; 2 – Gatithini; 3 – Chuka ***(-ve results)****; 4 – Marimanti; 5 – Ikumbo; 6 – Kathwana; 7 – Kaanwa; 8 – Mukothima* ***(-ve results)****; 9 – Magutuni* ***(-ve results)***

3.1.3 Genetic relatedness of duckweed species found in Tharaka-Nithi County

Sequence comparison results indicated that the duckweed samples represented several *Lemna* species, including *Lemna minor*, *Lemna turionifera*, *Lemna aequinoctialis*, and *Lemna perpusilla* (Table 3). Sample 1_RBCLA_Kathenya-RBCL-F showed 99.27% similarity to a *Lemna minor* voucher from China (GQ436374), and sample 5_RBCLA_Kathenya-RBCL-F displayed an identical match to the same voucher. Sample 2_RBCLA_Kathenya-RBCL-F shared the highest similarity (99.45%) with *Lemna minor* from Saudi Arabia (OK571369). Sample 4_RBCLA_Kathenya-RBCL-F was most similar (98.38%) to *Lemna turionifera* from China (NC_072328), suggesting intraspecific variation within the population. Sample 6_RBCLA_Kathenya-RBCL-F aligned closely (98.92%) with *Lemna aequinoctialis* from the USA (AY034228), while sample 7_RBCLA_Kathenya-RBCL-F matched *Lemna perpusilla* (98.38%) from the USA (AY034229). Overall, BLAST results demonstrated consistently high sequence similarities above 98%, confirming the presence of multiple *Lemna* species within the sampled sites. The consistent E-value of 0 across all samples supports the accuracy of molecular identification and highlights the genetic diversity within the duckweed populations analyzed.

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Table 3: BLAST search results showing high sequence similarity

SAMPLE ID	CLOSE RELATIVE	PERCENTAGE IDENTITY	E VALUE	ACCESSION NUMBER OF CLOSE RELATIVE	ORIGIN
1_RBCLA_Kathenya-RBCL-F	*Lemna minor voucher*	99.27	0	GQ436374	China
2_RBCLA_Kathenya-RBCL-F	*Lemna minor*	99.45	0	OK571369	Saudi Arabia
4_RBCLA_Kathenya-RBCL-F	*Lemna turionifera*	98.38	0	NC_072328	China
5_RBCLA_Kathenya-RBCL-F	*Lemna minor voucher*	99.27	0	GQ436374	China
6_RBCLA_Kathenya-RBCL-F	*Lemna aequinoctialis*	98.92	0	AY034228	USA
7_RBCLA_Kathenya-RBCL-F	*Lemna perpusilla*	98.38	0	AY034229	USA

Key: 1 – Chogoria; 2 – Gatithini; 4 – Marimanti 5 – Ikumbo; 6 Kathwana; 7 – Kaanwa.

3.1.4 Phylogenetic analysis of duckweed sequences

The phylogenetic tree (Figure 3) presents distinct clades of *Lemna* species, with *Azolla caroliniana* as the outgroup forming the first clade (bootstrap 1.3). The second clade groups *Lemna aequinoctialis* and *Lemna tenera* with RBCLA Kathenya-RBCL-F sequences (bootstrap 0.5). The third joins *Lemna turionifera* and *Lemna minor* (bootstrap 2.1). The fourth and final clades include *Lemna minor* and *Lemna perpusilla* in closely related clusters.

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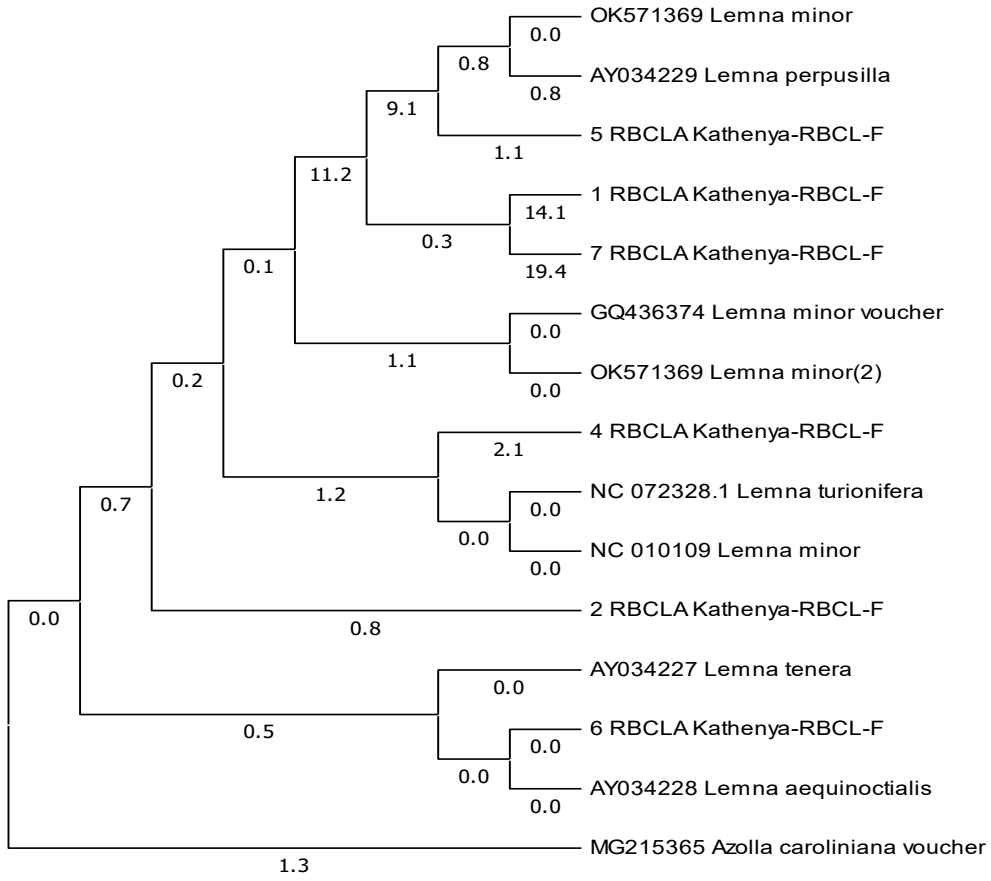

Figure 3: Phylogenetic tree. Isolates formed 5 distinct clades with sequences selected based on BLAST search results. Bootstrap values were based on 1000 replicates

3.1.5 Distribution of Duckweed species in Tharaka-Nithi County

The sampled habitats were located at altitudes ranging between 733 and 1626 meters, encompassing treated sewage ponds, wetlands with water-logged soils, and fishponds (Table 4). Sample 1_RBCLA_Kathenya-RBCL-F, collected from Chogoria at 1626 meters, was obtained from a treated sewage pond where duckweed formed dense surface mats. It was identified as *Lemna minor* voucher, characterized by dark green, obovate, asymmetric fronds lacking hyaline tissue, with a frond length (FL) of 5.6 mm, width (FW) of 2.27 mm, 2.67 nodes per colony, and 1.33 veins. Sample 2_RBCLA_Kathenya-RBCL-F from Gatithini (806 m) was collected from swampy wetlands near slow-moving streams and identified as *Lemna minor*. The fronds were dark green, symmetric, and had hyaline tissue, with FL 5.2 mm, FW 4.07 mm, 3.67 nodes, and 2.67 veins. Sample 4_RBCLA_Kathenya-RBCL-F from Marimanti (733 m) originated from fishponds and was identified as *Lemna turionifera*, featuring light green, asymmetric fronds with hyaline tissue, FL 5.8 mm, FW 3.53 mm, 3.67 nodes, and 3 veins. Sample 5_RBCLA_Kathenya-RBCL-F from Ikumbo (1208 m) was *Lemna minor* voucher, with symmetric, light green fronds (FL 5.84 mm, FW 5.33 mm, 3.33 nodes, 3.67 veins). Sample 6_RBCLA_Kathenya-RBCL-F from Kathwana (780 m) was *Lemna aequinoctialis*, with asymmetric, light green fronds (FL 5.8 mm, FW 4.6 mm, 3 nodes, 3 veins). Lastly, Sample

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7_RBCLA_Kathenya-RBCL-F from Kaanwa (1229 m) was *Lemna perpusilla*, having symmetric, light green fronds without hyaline tissue (FL 5.7 mm, FW 5.4 mm, 4 nodes, 1.33 veins).

Table 4: Distribution of Duckweed species in Tharaka-Nithi County

Sample ID	Location	Habitat	Species	Morphology
1_RBCLA_Kathenya-RBCL-F	Chogoria	Well-established treated sewage pond at an altitude of 1626 meters, latitude 0°13'56.6"S and Longitude 37°37'36.2"E. Duckweed forms a mat on the surface of the water	*Lemna minor voucher*	Obovate fronds with asymmetry, lacks hyaline tissue, and has a dark green color with rounded and extended features. It has a frond length (FL) of 5.6 mm, a frond width (FW) of 2.27 mm, 2.67 nodes per colony, and 1.33 veins.
2_RBCLA_Kathenya-RBCL-F	Gatithini	Wetland with swampy, water-logged soil and slow-moving freshwater streams. Duckweed occupies the sides of the streams. The altitude is 806 meters, latitude 0°3'17.6"N and longitude 37°59'45.9"E.	*Lemna minor*	Obovate fronds with symmetry and hyaline tissue. It is dark green, rounded, and not extended. It has an FL of 5.2 mm, an FW of 4.07 mm, 3.67 nodes per colony, and 2.67 veins.
4_RBCLA_Kathenya-RBCL-F	Marimanti	Located at 733 meters above the sea level, latitude 0°9'25.4"S and longitude 37°58'41.0"E. This site consists of well-established fish ponds where duckweed and other plants float on the water's surface.	*Lemna turionifera*	The duckweed in this area has obovate fronds with asymmetry and hyaline tissue. It is light green, rounded, and extended. It has an FL of 5.8 mm, an FW of 3.53 mm, 3.67 nodes per colony, and 3 veins.
5_RBCLA_Kathenya-RBCL-F	Ikumbo	Wetland with swampy, water-logged soil and small freshwater ponds. Duckweed and other plankton float on the surface. The site is located at 1208 meters above the sea level, latitude 0°16'19.7"S and longitude 37°44'5.6"E.	*Lemna minor voucher*	Obovate fronds with symmetry and hyaline tissue. It is light green, rounded, and extended. It has an FL of 5.84 mm, an FW of 5.33 mm, 3.33 nodes per colony, and 3.67 veins.
6_RBCLA_Kathenya-RBCL-F	Kathwana	Wastewater pond at 780 meters above the sea level. Co-ordinates were latitude 0°19'58.0"S and longitude 37° 52'12.8"E Duckweed occupies about a third of a well-established treated sewage pond	*Lemna aequinoctialis*	Obovate fronds with asymmetry and hyaline tissue. It is light green, rounded, and extended. It has an FL of 5.8 mm, an FW of 4.60 mm, 3 nodes per colony, and 3 veins.
7_RBCLA_Kathenya-RBCL-F	*Kaanwa*	1229 meters in altitude, latitude 0°19'35.0"S and longitude 37°43'15.9"E. Features;- well-established fish ponds with duckweed and other plankton floating on the surface.	*Lemna perpusilla*	Obovate fronds with symmetry, lacks hyaline tissue, and is light green with rounded and extended features. It has an FL of 5.7 mm, an FW of 5.40 mm, 4 nodes per colony, and 1.33 veins.

3.2 Discussion

The present study combined extensive fieldwork with detailed laboratory analysis to improve understanding of the identity, relative abundance, and distribution of duckweed species within the study area. Nine sampling sites were selected, from which a total of 144 duckweed samples were collected. These samples underwent morphological examination before molecular analysis and were grouped into nine distinct clones based on shared morphological traits. The morphological descriptors employed in the classification process included frond shape, symmetry, border, colour, apex form, venation, aerenchyma presence, frond length and width, the number of contiguous fronds, number of veins, frond area, frond length-to-width ratio, and the distance between frond base and root attachment. These descriptors have been effectively utilized in previous research to characterize duckweed species in Israel and Saudi Arabia (Al-Dakhil et al., 2021), where species belonging to *Lemna*, *Wolffia*, and *Spirodela* were successfully identified.

In this study, all nine clones displayed obovate fronds, rounded apices, and parallel venation, suggesting a consistent morphological framework. The variation observed among clones mainly stemmed from differences in symmetry, border characteristics, coloration, and measurements such as frond length and width. Despite these differences, the overall variability was relatively minor, which is consistent with the understanding that duckweed species exhibit limited morphological variation due to adaptation to aquatic environments with narrow ecological ranges (Les et al., 2002). This observation reinforces the notion that morphological traits alone are often insufficient for accurate species-level identification. Morphological features tend to be more useful in distinguishing genera rather than species within a genus (Andriani et al., 2019). Based on the morphological data, it was inferred that all nine clones likely belong to the genus *Lemna*, given their shared obovate shape, parallel venation, rounded apices, and predominantly asymmetrical fronds with hyaline tissue. Their frond lengths, which were consistently below 6 mm, further support this conclusion, aligning with the findings of Wang et al. (2010) and Ceschin et al. (2016), who observed that morphological differentiation among *Lemna* species is often minimal.

While the use of morphological descriptors provides a structured approach to classifying duckweed, it also highlights inherent limitations in relying solely on visible characteristics. Morphological identification requires significant expertise and can be challenging due to environmental influences that may alter plant appearance (Friedjung et al., 2022). Therefore, this study employed DNA barcoding as a complementary tool to improve species identification accuracy. Phylogenetic research combining morphological, anatomical, and molecular data has proven effective in classifying members of the Lemnaceae family, including *Lemna*, *Spirodela*, *Wolffia*, *Wolffiella*, and *Landoltia* (Ding et al., 2017). DNA barcoding, particularly when used with plastid genes such as *rbcL* and *matK*, has been shown to distinguish species with highly similar morphologies (Letsiou et al., 2024).

In this study, two chloroplast DNA regions, *rbcL* and *matK*, were used for molecular characterization of the nine duckweed clones. Prior studies have shown that these genes provide high amplification and sequencing success rates and have been recommended by the Consortium for the Barcode of Life (CBOL) as standard plant barcoding regions (Al-Dakhil et al., 2021). For instance, Wang et al. (2010) demonstrated that *matK* and *rbcL* achieved PCR success rates of 71% and 100%, respectively, for duckweed identification. These findings support the reliability of molecular barcoding in resolving taxonomic uncertainties in *Lemna* species (Bog et al., 2019).

The genus *Lemna* represents the largest and most complex group within the family Lemnaceae. Species differentiation within this genus has historically been problematic due to overlapping morphological traits and environmental plasticity (Crawford et al., 2005). Molecular tools such as *rbcL* barcoding have helped resolve these challenges, enabling researchers to identify specific *Lemna* species with greater confidence. For instance, Wang et al. (2010) identified eight *Lemna* species, noting clustering patterns that reflected evolutionary relationships. Similarly, Borisjuk et al. (2015) conducted extensive molecular studies across the genus, reinforcing the power of genetic data in refining *Lemna* taxonomy.

In this study, morphological and phylogenetic analyses revealed minimal variation among clones from different regions. All nine clones exhibited obovate frond shapes and rounded apices, with six clones showing light green coloration and hyaline borders. DNA barcoding confirmed that all clones belonged to the genus *Lemna*, supporting morphological observations. Specifically, clones from Chogoria, Ikumbo, and Gatithini were identified as *Lemna minor*, characterized by obovate fronds, rounded apices, and distinct hyaline margins. These results are consistent with previous findings by Azer (2013), who observed similar frond symmetry in *L. minor*, and with Al-Dakhil et al. (2021), who demonstrated that *rbcL* markers effectively identify this species. Clones from Chogoria and Ikumbo were further classified as *Lemna minor* subspecies voucher.

The distribution of duckweed species across habitats in Tharaka-Nithi County was influenced by environmental variables such as altitude, water quality, and habitat type. Samples were obtained from treated sewage ponds, fishponds, and swampy wetlands, all of which supported various *Lemna* species. For example, *Lemna minor* occurred in both Chogoria and Gatithini, showing slight differences in frond symmetry and hyaline tissue presence. *Lemna turionifera* was identified in fishponds at Marimanti, displaying light green fronds with hyaline borders. These differences reflect environmental adaptations within the same genus, emphasizing habitat-specific influences on morphology.

Duckweed species demonstrated exceptional adaptability to diverse freshwater environments, confirming their ecological importance in Tharaka-Nithi County. *Lemna minor*, observed in both treated sewage ponds and natural wetlands, showed resilience in stagnant and slow-flowing water. This adaptability indicates a broad ecological tolerance that enables the species to persist under varying hydrological conditions. Conversely, *Lemna turionifera* was primarily restricted to nutrient-rich, stagnant ponds, while *Lemna aequinoctialis* thrived in wastewater environments, confirming its preference for high-organic-content habitats. These findings align with Van der Plass (1972) and Andriani et al. (2019), who reported that duckweeds can survive not only in freshwater but also in slightly saline or muddy habitats.

The dominance of duckweed in multiple aquatic environments is supported by key biological traits. Its rapid growth allows it to quickly colonize new habitats and outcompete other aquatic plants. Its simple structure, composed mainly of fronds and roots, enhances nutrient uptake directly from the water, enabling survival in nutrient-poor and nutrient-rich systems alike (Ziegler et al., 2023). Additionally, duckweed displays strong phenotypic plasticity, adjusting morphology and physiology in response to environmental changes such as light intensity, temperature, and nutrient availability (Takacs et al., 2025). Its capacity for both sexual and asexual reproduction enhances adaptability and population expansion, contributing to its dominance in many aquatic ecosystems.

Ultimately, this study contributes to a deeper understanding of aquatic biodiversity in Tharaka-Nithi County by clarifying the distribution, genetic diversity, and ecological adaptability of *Lemna* species. Through the integration of morphological and molecular approaches, the research confirms the presence of multiple *Lemna* species across varied habitats and underscores their ecological significance. The findings emphasize that environmental parameters—such as altitude, water chemistry, and habitat structure—play critical roles in shaping duckweed distribution. Moreover, *Lemna* species provide essential ecosystem services, including nutrient cycling, carbon sequestration, and wastewater purification. Protecting natural wetlands and maintaining water quality are therefore vital for sustaining these valuable ecological functions. Finally, by demonstrating the efficiency of DNA barcoding in resolving taxonomic ambiguities, this study supports its continued use in biodiversity monitoring and conservation efforts across aquatic ecosystems.

4. Conclusion

i. Morphological analysis of duckweed collected from ponds and wetlands in Tharaka-Nithi County showed minimal variation among the nine clones. All exhibited obovate fronds with rounded apices, and only slight differences were observed in symmetry, color, and border, which were insufficient for species-level distinction.
ii. DNA barcoding using the *rbcL* gene confirmed that all samples belonged to the genus *Lemna*. The identified species included *Lemna minor*, *Lemna turionifera*, and *Lemna aequinoctialis*, indicating coexistence of multiple *Lemna* species within the study area.
iii. Sequence comparison suggested that duckweed distribution is influenced by altitude (733–1626 m) and habitat-specific water quality variations.

5. Recommendations

i. Based on the analysis of fourteen qualitative and quantitative morphological characteristics, the study identified nine distinct duckweed clones with only minimal variation among them. To enhance the accuracy of morphological identification and classification, it is recommended that additional features such as the prophyllum, dorsal meristem of emerging fronds, root tracheids, external locules, and number of stamens be included. Incorporating these traits will strengthen the precision of morphological studies and enable the identification of a wider range of duckweed clones in future research.
ii. The study demonstrated that DNA barcoding is a reliable tool for differentiating morphologically similar duckweed species. Hence, it is recommended that DNA barcoding be adopted as the primary approach for accurate species identification in future studies. The inclusion of *rbcL* and *matK* gene regions in molecular analysis will further improve the dependability of classification and minimize errors arising from morphological overlap.
iii. Findings revealed that duckweed can thrive in a variety of wetland environments, including both wastewater and freshwater systems. Therefore, maintaining environmental conditions that promote healthy growth and natural distribution is essential. This can be achieved through appropriate management practices tailored to specific habitat needs. Moreover, the ecological and purification value of duckweed in wastewater environments should be further explored and utilized.

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