RESEARCH ARTICLE AGROBIOLOGICAL RECORDS
ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

GENETIC DIVERSITY OF THE DUCKWEED FAMILY IN PAKISTAN

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ABSTRACT

Duckweeds (*Lemnaceae*) are among the smallest and fastest-growing aquatic flowering plants and are increasingly recognized for their ecological, nutritional, and biotechnological importance. Despite growing global interest, information on duckweed diversity and genetic structure in South Asia remains limited. In this study, we conducted the first integrative assessment of duckweed diversity, distribution, and chloroplast genetic variation across Pakistan using combined morphological and molecular approaches. A total of 142 duckweed field samples were collected from freshwater habitats across Punjab, Sindh, Khyber Pakhtunkhwa, and Baluchistan, yielding 232 axenic strains. From these, 53 representative isolates were selected for molecular analysis based on species representation, geographic coverage, and DNA quality. Morphological identification revealed four dominant species: *Lemna aequinoctialis*, *Spirodela polyrhiza*, *Wolffia borealis,* and *Wolffia globosa*, with *L. aequinoctialis* being the most widespread. Genetic diversity was assessed using two chloroplast DNA markers, the *rps16* intron and the *atpF–atpH* intergenic spacer. The *rps16* marker exhibited higher polymorphism and greater haplotype resolution than *atpF–atpH*, particularly within *L. aequinoctialis*, which showed multiple haplotypes and moderate nucleotide diversity. Regional analyses indicated elevated genetic diversity in southeastern Pakistan, while northwestern populations, composed mainly of *S. polyrhiza* and *W. globosa*, exhibited fewer haplotypes but greater nucleotide divergence, suggesting geographic isolation. Phylogenetic analyses confirmed species identities and demonstrated the superior resolving power of *rps16* for population-level differentiation. Overall, this study provides foundational insights into duckweed biodiversity and genetic structure in Pakistan and establishes a validated molecular framework for future ecological, evolutionary, and applied research on Lemnaceae in South Asia.

***Keywords*:** *Lemnaceae*, Chloroplast markers, Genetic polymorphism, Pakistan.

Article History (ABR-25-113) || Received: 27-Nov-2025 || Revised: 29-Dec-2025 || Accepted: 07-Jan-2026 || Published Online: 09-Jan-2026

1. INTRODUCTION

The duckweed family (*Lemnaceae*) includes some of the world's smallest and fastest-growing flowering plants, floating on or beneath the surface of freshwater bodies. Representatives of three genera, *Spirodela*, *Landoltia*, and *Lemna*, possess one or a few small roots emerging from their fronds, except a rootless species *Lemna trisulca*, whereas the remaining two genera, *Wolffiella* and *Wolffia*, are rootless and characterized by even smaller frond sizes. The five genera and 37 species, distributed worldwide except in polar regions (Bog et al., 2019). Different species of duckweed provide valuable opportunities for practical experiments and for exploring their physiology, biochemistry, and genetics (Fu et al., 2017; Zhou & Borisjuk, 2019; Fu et al., 2020; Baek et al., 2021). Their rapid growth makes them a cost-effective source of biomass for food and feed production (Han et al., 2022; Jaimes Prada et al., 2024). Duckweeds have attracted scientific interest due to their simplified morphology and ease of cultivation under controlled conditions. These traits make them valuable model organisms for research in plant physiology, nutrient metabolism, stress tolerance, and evolutionary adaptation in aquatic environments (Prada et al., 2024; Asniarti et al., 2025).

Duckweeds also serve as efficient natural filters, capable of treating a wide range of wastewater types (Zhou & Borisjuk, 2019). Their inherent ability to purify water can be further enhanced by selecting high-performing species or ecotypes and optimizing environmental conditions (Zhao et al., 2014; Ekperusi et al., 2019; Mustafa & Hayder, 2021; Kafle et al., 2022). Despite their small size, duckweeds exhibit high nutritional value, with balanced protein and essential amino acid composition (Prada et al., 2024). Key factors such as nutrient ratios, light intensity, and frond density significantly influence their efficiency, positioning duckweeds as valuable tools in sustainable and eco-friendly water treatment strategies (Walsh et al., 2020; Walsh et al., 2021a; 2021b). Duckweeds exhibit highly efficient nitrogen uptake and internal recycling mechanisms (N), which is an important nutrient for helping plants

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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grow and build biomass. Duckweeds exhibit highly efficient nitrogen uptake and internal recycling mechanisms, enabling sustained growth under nitrogen-limited conditions (Guo et al., 2020).

Duckweeds exhibit high nutritional value, with protein contents ranging from 16–42%, and provide all essential amino acids required for human nutrition (Xu et al., 2023). Duckweed species *L.minor* can contain around 40% protein (Escobar & Escobar, 2017). Duckweed protein provides all the amino acids the human body needs. It includes all nine essential amino acids (EAAs) as well as several non-essential ones. The levels of each amino acid in duckweed meet the WHO requirements (Xu et al., 2015; Appenroth et al., 2017). The starch content of *S. polyrhiza* has been further analysed and compared to that of corn starch. *S. polyrhiza* contains 23.3% starch on a dry weight basis, which is lower than the 66.5% starch content observed in B73 corn (Lee et al., 2016). The high biomass production resulting from their useful uses in biotechnology, water treatment, feed, and food (Appenroth et al., 2017; Iqbal et al., 2019; Sońta et al., 2019). *S. polyrhiza* exhibits the highest carbohydrate (34.5%) and fiber (14.5%) contents, whereas *W. globosa* demonstrates superior protein (39.6%) and fat (7.5%) levels (Xu et al., 2023). The ash content of *W. arrhiza* (17.9%) is higher than that of *L.minor* (13.3%), the latter of which also possesses notable levels of fiber (14%) and protein (38.3%) (Ullah et al., 2021). Duckweed has been found to yield an average of 2,080kg of protein per hectare annually, making it a promising alternative protein source. This yield is significantly more than that of traditional crops like maize (179kg ha^{-1} $year^{-1}$), soybeans (303kg ha^{-1} $year^{-1}$) and nuts (229kg ha^{-1} $year^{-1}$) (Sosa et al., 2024).

With the advancement of molecular taxonomy, molecular fingerprinting and sequencing have become central methods of identification (Bog et al., 2013). The principle of DNA-based molecular identification involves analysing polymorphisms in specific non-coding intron and gene spacer regions, with a primary focus on the chloroplast genome (Tippery & Les, 2020). Duckweed genetics, molecular evolution, and diversity are becoming increasingly popular due to their numerous applications. Duckweeds have 14-fold different genome sizes; the great duckweed (*S. polyrhiza* L.) has a genome of 160 Mb, while *W. arrhiza* L. has a genome of about 2.2 Gb (Sree et al., 2016). Applications of DNA barcoding include identifying microorganisms, recognizing species lacking distinctive morphological traits, and detecting taxa from environmental samples (Ali et al., 2014). Through advanced techniques such as DNA barcoding and the study of the genetic blueprints of chloroplasts and mitochondria, scientists have begun to unravel the mysteries of duckweed diversity. Genomic analyses of *S. polyrhiza* indicate extensive genome streamlining, with reduced gene family sizes and loss of non-essential genes associated with its free-floating aquatic growth habit and simplified morphology. Meanwhile, deep RNA sequencing has revealed contrasting stress-response strategies, with *Spirodela* entering dormancy and *Landoltia* exhibiting transcriptional responses to nutrient limitation (Wang & Messing, 2015).

Duckweeds typically colonize stagnant or slow-moving freshwater habitats and enabling them to adapt efficiently to diverse environmental conditions (Baek et al., 2021). *Spirodela* exhibited the highest specific growth rate (0.21 day^{-1}), whereas *Lemna* and *Wolffia* showed comparatively lower but similar rates (0.18 day^{-1} each) (Faizal et al., 2021). Interest in duckweed research has increased over time, with an initial rise in scientific publications occurring around the early 2000s, reflecting growing recognition of duckweeds as valuable experimental and applied systems (Fig. 1). A second and more pronounced increase has occurred since approximately 2015–2016, coinciding with advances in molecular biology, genomics, and biotechnology. Studies focusing specifically on duckweed biodiversity and genetic diversity expanded substantially during this later period.

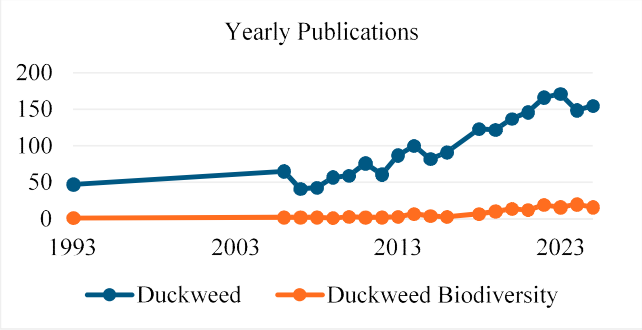

Fig. 1: Scientific publications from 1993 to 2025 matching the keywords "duckweed" and "duckweed biodiversity" from PubMed https://pubmed.ncbi.nlm.nih.gov/ accessed on 20 December 2025.

Chloroplast phylogenomic research has provided new insights into *Lemnaceae* evolution but has largely excluded the South Asian flora. Therefore, investigating Pakistan's duckweed populations presents a unique opportunity to examine how climatic diversity and potential evolutionary isolation influence the genetic structure of duckweeds in this understudied region (He et al., 2025). Despite the global importance of duckweeds and the increasing number of molecular studies in Europe, East Asia, and North America, duckweed diversity in South Asia remains poorly documented. In particular, Pakistan represents a major geographic gap in current *Lemnaceae* distributional and genetic datasets. To date, no integrative study combining extensive field sampling, axenic culture establishment, morphological identification, and chloroplast DNA barcoding has been conducted for duckweeds in this region. The present study addresses this

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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gap by providing the first comprehensive assessment of duckweed species composition, geographic distribution, and genetic diversity in Pakistan. Therefore, this study provides the first integrative assessment of duckweed species diversity, geographic distribution and chloroplast genetic variation across Pakistan.

In the present paper, we report on the distribution and biodiversity of duckweed species in Pakistan. Duckweed samples were obtained from various sites from Pakistan and grown under sterile in vitro conditions. The genetic profiles were identified through sequencing and analysis of two chloroplast markers: the ribosomal protein S16 intron (*rps16*) and the *atpF-atpH* intergenic spacer region.

2. MATERIALS AND METHODS

2.1. Collection of Duckweed Samples

Duckweed samples were collected during multiple field excursions across Punjab, Sindh, Khyber Pakhtunkhwa (KPK) and one site in Baluchistan, Pakistan. A total of 142 duckweed field samples were collected from freshwater habitats across Pakistan. Each field sample was processed independently and, depending on species composition and colony structure, yielded one or more viable duckweed strains. In total, 232 axenic duckweed strains were successfully isolated and morphologically identified. Immediately after collection, plants were gently rinsed to remove debris, individually wrapped in pre-moistened sterile wipes, enclosed in resealable plastic bags, and labelled with site-specific information to preserve sample integrity during transport. For cultivation, duckweed samples were surface-sterilized and maintained under axenic conditions in vitro using a modified half-strength E-medium. The medium contained defined concentrations of macronutrients, micronutrients, and chelating agents and was sterilized prior to inoculation. Sterile duckweed plants were then transferred aseptically into the prepared medium and maintained under controlled growth conditions for subsequent morphological and molecular analyses. Genetic diversity was analysed by sequencing two chloroplast DNA markers, the *rps16* intron and the *atpF-atpH* spacer.

2.2. Cultivation of Duckweed Clones

Duckweeds were cultivated in a modified half-strength E-medium, a commonly used nutrient medium for *Lemnaceae*. E-Stock 1 (KNO_3), E-Stock 2 [$Ca(NO_3)_2 \cdot 4H_2O$], E-Stock 3 (KH_2PO_4 and $MgSO_4 \cdot 7H_2O$), E-Stock 4 (trace elements: H_3BO_3, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $Na_2MoO_4 \cdot 2H_2O$), and E-Stock 5 (iron chelate: $FeCl_3 \cdot 6H_2O$ and Na_2EDTA) were prepared separately. The working medium was obtained by diluting 10mL each of E-Stocks 1, 2, 3, and 5(100X) and 1mL of E-Stock 4(1000X) into 2000mL of distilled water, followed by sterilization at 121°C for 15min. After cooling, the medium was aseptically dispensed into sterile culture vessels. Prior to inoculation, duckweed plants were surface-sterilized by immersion in 1% sodium hypochlorite for 1–2min followed by repeated rinsing with sterile distilled water. Surface sterilization was confirmed by repeated subculturing and microscopic inspection to ensure axenic conditions prior to morphological and molecular analyses. A single sterile frond from each culture was then transferred aseptically into the prepared E-medium and maintained under controlled environmental conditions.

2.3. Morphological and Molecular Analysis

Morphological classifications were performed using size, shape, color, number of veins and number of roots in the frond. From 142 samples, 53 representative isolates were selected for molecular analyses. Selection was based on (i) species representation to include all morphologically distinct taxa, (ii) geographic coverage to ensure representation of major ecological zones and (iii) DNA quality, including vigorous growth and absence of visible contamination. Total DNA was extracted using a Rapid Plant Genomic DNA Isolation Kit (TianGen, Beijing, China).

Table 1: PCR Reaction Mixture Composition

Component	Volume (µL)
2x Taq PCR master mix with dye (Tiangen, KT211)	19
Forward Primer	2
Reverse Primer	2
DNA Template	2
Nuclease-free water	25
Total Volume	50

DNA fragments of the non-coding spacer *atpF-atpH* and the chloroplast ribosomal protein S16 gene intron (*rps16*) were amplified by polymerase chain reaction (PCR) as previously described (Xu et al., 2015). Details of the PCR reaction mixture composition are provided in Table 1. The *rps16* was amplified using the primers *rps16 F* (5′AAA CGA TGT GGT ARA AAG CAA C 3′) and *rps16 R* (5′AAC ATC WAT TGC AAS GAT TCG ATA 3′). The PCR conditions were pre-denatured at 94°C for 4min, followed by 30 cycles at 94°C, 30s; 58°C, 45s; 72°C, 1min, and a further extension at 72°C for 7min. The primers used to amplify *atpF-atpH* was *HNP307 F* (5′-ACT CGC ACA CAC TCC CTT TCC-3′) and *HNP308 R* (5′-GCT TTT ATG GAA GCT TTA ACA AT-3′). The reaction conditions were pre-denatured at 94°C for 4min, followed by 30 cycles of 94°C, 30s; 53°C, 45s; 72°C, 1min, and a further extension at 72°C for 7min. The GenBank accession numbers of the *rps16* and *atpF-atpH* sequences are listed in Table 2.

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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Table 2: Duckweed strains with *atpF-atpH* and *rps16* sequenced in this study

Strain	Species	Coordinates	City	GenBank Accession	
				rps16	*atpF-atpH*
PAK0202	*Lemna aequinoctialis*	29°51′4.17″N, 71°20′7.92″E	Multan	PX437826	PX442439
PAK0302	*Lemna aequinoctialis*	29°51′8.02″N, 71°20′5.18″E	Multan	PX437827	PX442440
PAK0502	*Lemna aequinoctialis*	30°10′23.12″N, 71°20′52.22″E	Multan	PX437828	PX442441
PAK0702	*Lemna aequinoctialis*	30°12′23.10″N, 71°22′28.08″E	Multan	PX437829	PX442442
PAK1002	*Lemna aequinoctialis*	30°35′10.17″N, 73°16′30.06″E	Lodhran	PX437830	PX442443
PAK1902	*Lemna aequinoctialis*	31°44′5.19″N, 72°40′51.24″E	Chiniot	PX437832	PX442445
PAK2102	*Lemna aequinoctialis*	30°32′16.48″N, 72°57′26.24″E	Chichawatni	PX437833	PX442446
PAK2402	*Lemna aequinoctialis*	30°38′17.40″N, 72°50′5.77″E	Sahiwal	PX437834	PX442447
PAK2502	*Lemna aequinoctialis*	30°35′55.17″N, 72°40′51.24″E	Sahiwal	PX437835	PX442448
PAK2802	*Lemna aequinoctialis*	31°24′51.83″N, 73°5′53.84″E	Faisalabad	PX437838	PX442451
PAK3002	*Lemna aequinoctialis*	31°32′16.27″N, 73°12′55.88″E	Faisalabad	PX437839	PX442452
PAK3102	*Lemna aequinoctialis*	31°32′23.66″N, 73°12′53.96″E	Faisalabad	PX437840	PX442453
PAK4002	*Lemna aequinoctialis*	31°36′17.48″N, 74°17′40.97″E	Lahore	PX437843	PX442456
PAK4202	*Lemna aequinoctialis*	31°39′12.03″N, 73°54′56.49″E	Sheikhupura	PX437844	PX442457
PAK4302	*Lemna aequinoctialis*	31°36′10.30″N, 73°53′44.02″E	Sheikhupura	PX437845	PX442458
PAK4602	*Lemna aequinoctialis*	32°3′16.44″N, 74°10′52.04″E	Gujranwala	PX437846	PX442459
PAK9501	*Lemna aequinoctialis*	32°2′34.22″N,73°41′36.55″E	Hafizabad	PX437849	PX442462
PAK10202	*Lemna aequinoctialis*	24°56′35″N, 68°04′17″E	Thatta	PX437853	PX442466
PAK10501	*Lemna aequinoctialis*	26°25′25″N, 67°52′04″E	Sehwan	PX437854	PX442467
PAK10702	*Lemna aequinoctialis*	26°44′14″N,67°47′19″E	Dadu	PX437856	PX442469
PAK10801	*Lemna aequinoctialis*	24°51′34″N, 67°9′46″E	Karachi	PX437857	PX442470
PAK11201	*Lemna aequinoctialis*	27°59′ 47″N,63°15′41″E	Kolpur	PX437859	PX442472
PAK11402	*Lemna aequinoctialis*	24°51′58″N, 67°10′02″E	Karachi	PX437861	PX442474
PAK11502	*Lemna aequinoctialis*	25°07′37.34″N, 67°17′49″E	Karachi	PX437862	PX442475
PAK11602	*Lemna aequinoctialis*	24°58′19.74″N, 67°01′26.76″E	Karachi	PX437863	PX442476
PAK11702	*Lemna aequinoctialis*	24°56′44.47″N, 66°45′47.06″E	Karachi	PX437864	PX442477
PAK12001	*Lemna aequinoctialis*	24°58′02.54″N , 67°18′ 42.18″E	Karachi	PX437866	PX442479
PAK12102	*Lemna aequinoctialis*	24°56′22.71″N, 67°17′24.56″E	Karachi	PX437867	PX442480
PAK1601	*Spirodela polyrhiza*	31°2′36.38"N, 72°58′58.47"E	Samundri	PX437831	PX442444
PAK2601	*Spirodela polyrhiza*	31°26′12.25″N, 73°7′44.54″E	Faisalabad	PX437836	PX442449
PAK2801	*Spirodela polyrhiza*	31°24′51.83″N, 73°5′53.84″E	Faisalabad	PX437837	PX442450
PAK3301	*Spirodela polyrhiza*	31°41′12.00″N, 74°5′44.56″E	Sheikhupura	PX437841	PX442454
PAK3601	*Spirodela polyrhiza*	31°30′41.89″N, 74°17′18.10″E	Lahore	PX437842	PX442455
PAK10001	*Spirodela polyrhiza*	25°07′04″N, 68°32′21"E	Tando Muhammad Khan	PX437850	PX442463
PAK10101	*Spirodela polyrhiza*	25°07′37"N, 68°32′26"E	Tando Muhammad Khan	PX437851	PX442464
PAK10201	*Spirodela polyrhiza*	24°56′35″N, 68°04′17″E	Thatta	PX437852	PX442465
PAK10701	*Spirodela polyrhiza*	26°50′14″N, 67°21′55″E	Dadu	PX437855	PX442468
PAK12401	*Spirodela polyrhiza*	34°11′34.93"N, 72°9′27.77"E	Garhi kapura	PX437868	PX442481
PAK12501	*Spirodela polyrhiza*	34°0′42.53"N, 72°1′2.31"E	Nowshera	PX437869	PX442482
PAK12801	*Spirodela polyrhiza*	31°50′29.84"N,70°55′33.14"E	Dera Ismail Khan	PX437870	PX442483
PAK13601	*Spirodela polyrhiza*	34°2′38.34"N, 71°53′32.05"E	Kheshgi	PX437873	PX442486
PAK13801	*Spirodela polyrhiza*	32°58′33.72"N, 70°39′53.37"E	Banu	PX437875	PX442488
PAK14101	*Spirodela polyrhiza*	32°37′12.84"N, 70°53′47.65"E	Lucky marwat	PX437877	PX442490
PAK6901	*Wolffia borealis*	32°4′44.79"N, 73°44′3.19"E	Hafizabad	PX437847	PX442460
PAK8002	*Wolffia borealis*	32°17′54.42"N, 74°22′1.98"E	Daska	PX437848	PX442461
PAK10902	*Wolffia borealis*	25°32′0.02″N, 66°36′36.67″E	Karachi	PX437858	PX442471
PAK11302	*Wolffia borealis*	27°59′47″N, 63°15′41″E	Karachi	PX437860	PX442473
PAK11802	*Wolffia borealis*	25°34′18.51"N, 67°04′43.96"E	Karachi	PX437865	PX442478
PAK13002	*Wolffia globosa*	31°50′1.36"N, 70°55′44.3"E	Banu	PX437871	PX442484
PAK13203	*Wolffia globosa*	33°35′34.44"N, 71°23′7.78"E	Kohat	PX437872	PX442485
PAK13702	*Wolffia globosa*	33°55′47.14"N, 72°4′58.69"E	Nowshera	PX437874	PX442487
PAK13802	*Wolffia globosa*	32°58′33.72"N, 70°39′53.37"E	Banu	PX437876	PX442489
PAK14203	*Wolffia globosa*	32°37′12.84"N, 70°53′47.65"E	Lucky marwat	PX437878	PX442491

Genetic diversity was evaluated by aligning the *rps16* and *atpF-atpH* chloroplast sequences through the ClustalW alignment program (Thompson et al., 2003), followed by analysis with DnSP6 software (Rozas et al., 2017). The haplotype diversity, nucleotide diversity and the theta-w per site were estimated using the same default parameters of the software in all cases (Rozas et al., 2017).

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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2.4. Data Analysis and Quality Control

Raw sequence chromatograms were imported into MacVector v18.2 (MacVector, Inc., USA) for trimming, editing, and quality inspection. Low-quality regions, primer residues, and ambiguous bases were manually removed, and forward and reverse reads were assembled into consensus sequences. Any sequences containing more than 2% ambiguous bases or unresolved peaks were re-sequenced to ensure accuracy. Multiple sequence alignments were generated in MacVector using the integrated ClustalW algorithm with the following parameters: gap opening penalty = 15.0, gap extension penalty = 6.66, and delay divergent cutoff = 30%. Alignments were subsequently exported to MEGA11 (Tamura et al., 2021) for evolutionary analysis. The Tamura–Nei model (Tamura & Nei, 1993) was applied to estimate nucleotide substitutions, and phylogenetic trees were reconstructed using Neighbor-Joining (NJ) with 1,000 bootstrap replications to assess clade support. Genetic diversity indices, including haplotype diversity (Hd), nucleotide diversity (π), and θw per site, were calculated using DnaSP v6.12.03 (Rozas et al., 2017). All final sequences were verified against GenBank via BLASTn to confirm species identity prior to inclusion in phylogenetic analysis.

2.5. DNA Barcoding Analysis

Reference sequences of *rps16* and *atpF-atpH* used for DNA barcoding and tree-based analyses were obtained from GenBank. The final trees included 37 reference *rps16* sequences and *atpF-atpH* sequences. Sequences were aligned with the help of ClustalW (Thompson et al., 2003) and the alignment results were imported to Mega11 (Tamura et al., 2021). The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura & Nei, 1993). All positions containing gaps and missing data were eliminated (complete deletion option). There was a total of 491 positions in the final *rps16* dataset and 374 positions in the final *atpF-atpH* dataset. Evolutionary analyses were conducted in MEGA11 software (Tamura et al., 2021). Strain identifiers consist of the prefix "PAK" (Pakistan) followed by a numeric code corresponding to the field collection and isolate number; each identifier represents a unique axenic duckweed line derived from a single sampling location.

3. RESULTS

3.1. Distribution of Duckweed Species in Pakistan

A total of 142 duckweed field samples were collected from freshwater habitats across Pakistan, yielding 232 isolated strains. Based on their morphological characteristics, these field strains were identified as belonging to four distinct species across three genera within the family *Lemnaceae* (Fig. 2). Most samples were collected in Punjab**,** where *L. aequinoctialis* was widespread across canals, agricultural ponds, and floodplain water bodies. *S. polyrhiza* was also frequent in Punjab and often co-occurred with *L. aequinoctialis* and *W. borealis*. In Sindh, *L. aequinoctialis* and *S. polyrhiza* dominated and occasional findings of *W. borealis*. Collections from Khyber Pakhtunkhwa (KPK) consisted mainly of *S. polyrhiza* and *W. globosa* which occurred primarily in irrigation ponds and agricultural fields, generally as single-species populations. In Baluchistan**,** only one site was sampled, where a localized duckweed population was documented, highlighting the limited records available from this province (Fig. 3).

Fig. 2: Duckweed community in Pakistan: Field photographs of duckweed collections and species diversity observed across different regions of Pakistan.

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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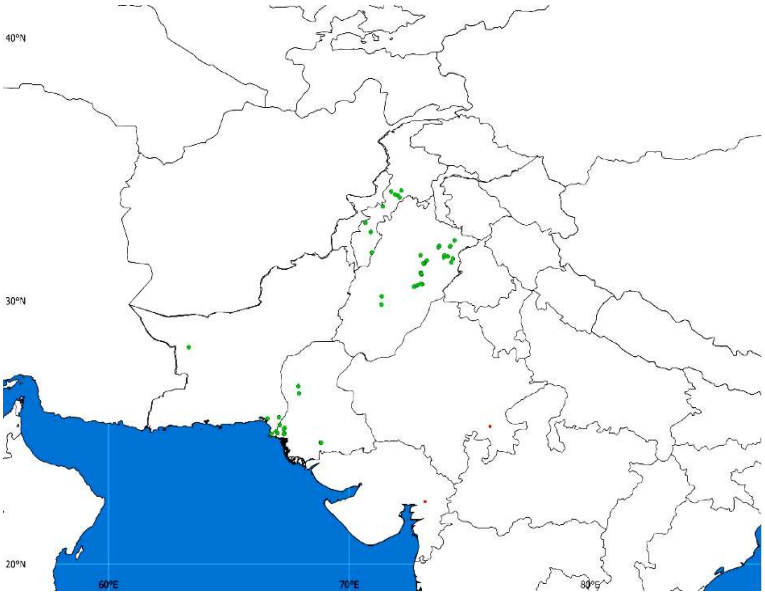

Fig. 3: Distribution of duckweed samples in Pakistan.

3.2. Morphological Analysis

Across the surveyed sites, clear differences were observed in the spatial occurrence and community structure of the recorded duckweed species (Fig. 4). *L. aequinoctialis* showed the broadest ecological amplitude, occurring in both managed and natural freshwater systems and persisting across a wide geographic range. In contrast, *S. polyrhiza* displayed a patchier distribution pattern and was frequently encountered in association with other duckweed taxa rather than as an isolated population. Species of *Wolffia* genus were comparatively rare and exhibited localized occurrence, suggesting limited dispersal or more specific habitat requirements. *W. globosa* was typically detected in mixed assemblages, whereas *W. borealis* was restricted to a small number of isolated sites. The absence of extensive single-species stands of *Wolffia* and their confinement to mixed communities indicate narrower ecological tolerance relative to the more widespread *Lemna* and *Spirodela* taxa (Table 3).

3.3. Genetic Diversity of duckweed Clones based on rps16 and atpF-atpH

The chloroplast *rps16* intron and *atpF-atpH* fragments of 53 representative clones, including 28 clones of *L. aequinoctialis*, 15 clones of *S. polyrhiza* and 5 clones of *W. globosa and* 5 clones of *W. borealis* were amplified and sequenced. The genetic diversity assessment of *L. aequinoctialis* across Pakistan, based on two chloroplast markers (*rps16* and *atpF–atpH*), revealed notable variation in genetic composition (Table 4). The *rps16* marker exhibited a higher number of both indels (6) and SNPs (12) compared to *atpF–atpH*, which had 2 indels and 7 SNPs. Correspondingly, *rps16* also displayed greater haplotype richness, with 7 haplotypes and a diversity index of 0.648, suggesting a broader genetic base. Nucleotide diversity (π) for *rps16* was slightly lower at 0.00364 than *atpF–atpH* (0.00462), indicating moderate levels of sequence variation. The overall values for Theta-W per site further confirmed balanced polymorphism within populations, showing 0.00307 and 0.00263 for *rps16* and *atpF–atpH*, respectively. These results collectively highlight a reasonable degree of intra-species variation in *L. aequinoctialis* populations across Pakistan.

Table 3: Morphological identification of duckweed strains collected in Pakistan

Species	Morphology	Locations	Strains
Lemna aequinoctialis	Fronds flattened, 2–3.5 mm long, obovate, asymmetrical at basal end; floating on water surface; one root per frond; dorsal surface with median papillae and three veins; two lateral pouches at basal end.	Widely distributed in Punjab, Sindh and Khyber Pakhtunkhwa (KPK), in canals, and River's floodplain ponds.	112
Spirodela polyrhiza	Fronds wide-obovate, 5–8 mm long, 4–6 mm wide; flat, green on both surfaces in observed strains; bearing 7–21 roots per frond, one or two perforating the paraphylum; two lateral pouches at basal end	Common in Punjab, Sindh, and Khyber Pakhtunkhwa (KPK), including River floodplain ponds.	95
Wolffia globosa	Rootless; fronds globoid or ovoid, flat-topped, 0.5–1.5 mm in diameter; one lateral pouch; one daughter plant produced at a time, rounder and more swollen, almost pear-shaped. The budding part is bigger and more inflated,	Found in Punjab and Khyber Pakhtunkhwa, including Balar Stream (Garhi, Kapura), Gujranwala canals, Gulderi Dam (Noshera), and Upper Chenab Canal.	15
Wolffia borealis	Long and narrow with a smooth surface. Streamlined and has a small, clear budding part at one end, giving it a slender appearance, Rootless; very small ellipsoid fronds; morphologically like *W. globosa* but distinct genetically; one lateral pouch; one daughter plant produced at a time.	Found in Hafizabad Daska and Karachi	10

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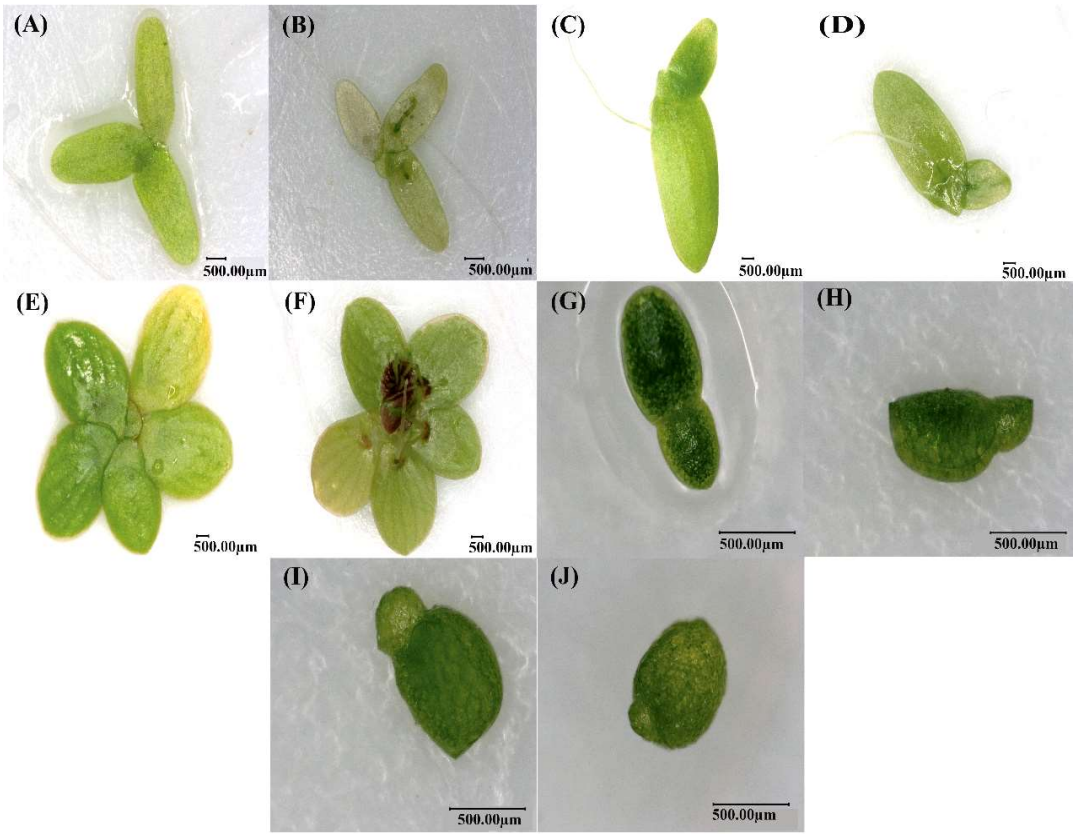

Fig. 4: Morphological diversity among selected duckweed species observed under a stereomicroscope. (A–D) *L. aequinoctialis* showing characteristic elliptical to trilobed fronds with single root structures and visible budding sites. (E–F) *S. polyrhiza* is distinguished by broader, clustered fronds and multiple visible roots. (G–H) *W. borealis*, a rootless species with spherical to ovoid fronds and lateral budding. (I–J) *W. globosa* also rootless, with bilobed or boat-shaped fronds indicative of vegetative propagation. All images captured under uniform magnification; scale bars = 500 µm.

Table 4: Genetic diversities of *L. aequinoctialis* in Pakistan

	L. aequinoctialis	
	rps16	*atpF–atpH*
Indels	6	2
SNPs	12	7
Haplotype number	7	2
Haplotype diversity	0.648	0.452
Nucleotide diversity	0.00364	0.00462
Theta-w per site	0.00307	0.00263

A regional comparison of L. aequinoctialis populations revealed distinct patterns of genetic diversity between eastern and southeastern regions of Pakistan (Table 5). In the *rps16*, haplotype diversity was marginally higher in the southeastern population (0.473) than the eastern (0.426), with a corresponding increase in nucleotide diversity (0.00358 vs. 0.00127). A similar trend was observed in the *atpF–atpH* region, where the southeastern region exhibited considerably greater diversity, both in haplotype (0.436 vs. 0.118) and nucleotide diversity (0.00446 vs. 0.00120). Notably, the average nucleotide differences per kilobase were also significantly higher in the southeast for both markers, implying more frequent sequence variation. These patterns suggest that southeastern populations harbor more complex genetic structures, possibly influenced by diverse ecological conditions or broader gene flow from surrounding regions. When comparing duckweed populations at the family level across regions, Table 6 presents clear regional differences in genetic diversity. In the eastern and southeastern parts, high haplotype diversity and nucleotide variability were observed for both *rps16* and *atpF–atpH*, suggesting richer intra-family variation. In northwestern Pakistan, only *S. polyrhiza* and *W. globosa* were recorded, showing moderate haplotype diversity values (0.530 for *rps16* and 0.545 for *atpF-atpH*), yet exhibited the highest nucleotide diversity in *rps16* (0.07905) among all regions. This finding indicates that while the number of unique haplotypes is fewer in the northwest, the genetic distances among them are greater, particularly in *rps16*. This could reflect long-term divergence or limited gene flow in this area. The increased nucleotide differences per kilobase (76.364 for *rps16* and 27.273 for *atpF–atpH*) further reinforce the presence of substantial intra-lineage variation despite the limited number of taxa. Overall, the northwest appears genetically distinct, shaped by its exclusive species composition and possibly geographic isolation.

Citation: Imtiaz U, Shakoor K, Wang X, Liu K, Tan D and Zhang J, 2026. Genetic diversity of the duckweed family in Pakistan. Agrobiological Records 23: 51-64. https://doi.org/10.47278/journal.abr/2026.004

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Table 5: Comparison of genetic diversities of *L. aequinoctialis* populations in the east and southeast parts of Pakistan

	rps16		*atpF-atpH*	
	East	South-east	East	South-east
Haplotype diversity	0.426	0.473	0.118	0.436
Nucleotide diversity	0.00127	0.00358	0.00120	0.00446
Theta-w per site	0.00265	0.00340	0.00302	0.00349
Nucleotide differences per kb	1.272	3.600	0.824	3.055

Table 6: Comparison of genetic diversities between the duckweed populations in the eastern, northwestern and southeastern parts of Pakistan on family level

		rps16			*atpF-atpH*	
	East	North-west	South-east	East	North-west	South-east
Haplotype diversity	0.643	0.530	0.752	0.525	0.545	0.739
Nucleotide diversity	0.061	0.079	0.078	0.033	0.039	0.045
Theta-w per site	0.054	0.049	0.053	0.032	0.025	0.035
Nucleotide differences per kb	55.39	76.36	71.51	22.28	27.27	30.72

3.4. Tree-based Identification of Species Considering rps16 and atpF-atpH Sequences

Phylogenetic analyses based on the chloroplast *atpF-atpH* intergenic spacer were first conducted to assess species-level relationships among the selected duckweed isolates (Fig. 5A). The Neighbor-Joining (NJ) tree resolved the samples into distinct, well-supported species-specific clades**,** each corresponding to reference sequences retrieved from GenBank (Fig. 5B). All *L. aequinoctialis* isolates clustered tightly with the reference strain *L. aequinoctialis* DW0101-3, supported by high bootstrap values (100%), confirming their taxonomic identity. However, intraspecific resolution within *L. aequinoctialis* was limited, with only two haplotypes detected, reflecting the relatively low sequence polymorphism of the *atpF–atpH* spacer.

Similarly, all *S. polyrhiza* accessions formed a single, highly supported clade together with the reference strain DW0202-3, showing no detectable intraspecific divergence at this locus. *W. globosa* and *W. borealis* isolates clustered consistently with their respective reference sequences, however, these two species failed to form clearly separated and well-supported clades in the *atpF–atpH* tree (Fig. 5B). To improve visualization of this clade, an enlarged subtree derived from the same *atpF–atpH* phylogenetic reconstruction (Fig. 5C), still does not represent a separate analysis, but rather a magnified view of the same dataset and topology, therefore, *atpF–atpH* alone cannot tell *W. globosa* and *W. borealis* from each other.

Phylogenetic reconstruction using the chloroplast *rps16* intron provided higher resolution and revealed greater intraspecific variation (Fig. 6). All *L. aequinoctialis* isolates again clustered with the reference strain DW0101-3; however, in contrast to *atpF–atpH*, multiple distinct haplotypes were clearly resolved within this clade. This pattern corresponds with the higher number of SNPs, indels, and increased haplotype diversity detected for *L. aequinoctialis* in the *rps16* diversity analyses, indicating substantial chloroplast genetic variation within this species.

S. polyrhiza accessions formed a compact, strongly supported clade with minimal internal branching, confirming the low level of chloroplast genetic diversity observed for this species. In contrast, *Wolffia* species were distinctly resolved, with *W. globosa* and *W. borealis* each forming separate, well-supported clades consistent with their reference sequences.

As with the *atpF–atpH* analysis*, rps16* phylogeny presents a focused visualization of the *Wolffia* clade, extracted from the complete tree to clearly illustrate interspecific separation and branching patterns within this genus (Fig. 6). This magnified view highlights the superior resolving power of the *rps16* marker for distinguishing closely related taxa and detecting finer-scale genetic structure.

Overall, the congruent topologies obtained from both chloroplast markers confirm the morphological and taxonomic identification of duckweed species collected from Pakistan. However, the greater sequence variation and higher phylogenetic resolution provided by *rps16* highlight its value as a robust primary barcode marker for assessing genetic diversity and evolutionary relationships within the family *Lemnaceae*, whereas atpF–atpH remains useful for rapid, reliable species-level identification.

4. DISCUSSION

This study reveals that duckweed populations in Pakistan are not genetically uniform and exhibit pronounced differences in diversity across taxa and regions. Among the four species examined *L. aequinoctialis*, *S. polyrhiza*, *W. borealis*, and *W. globosa*, *L. aequinoctialis* exhibited significant chloroplast genetic variation, with multiple haplotypes, indels, and SNPs, particularly in southeastern populations. In contrast, *S. polyrhiza* and both *W. borealis and W. globosa* species were genetically invariant for the chloroplast loci studied, indicating clonal or low-diversity populations. The *rps16* intron proved more informative than the *atpF–atpH* spacer for detecting intraspecific polymorphism. These findings underscore that morphological simplicity and asexual reproduction in duckweeds do not imply genetic homogeneity. Instead, different species follow divergent evolutionary trajectories, and genetic diversity is distributed unevenly across Pakistan′s landscapes. The contrast between genetically rich *L.*

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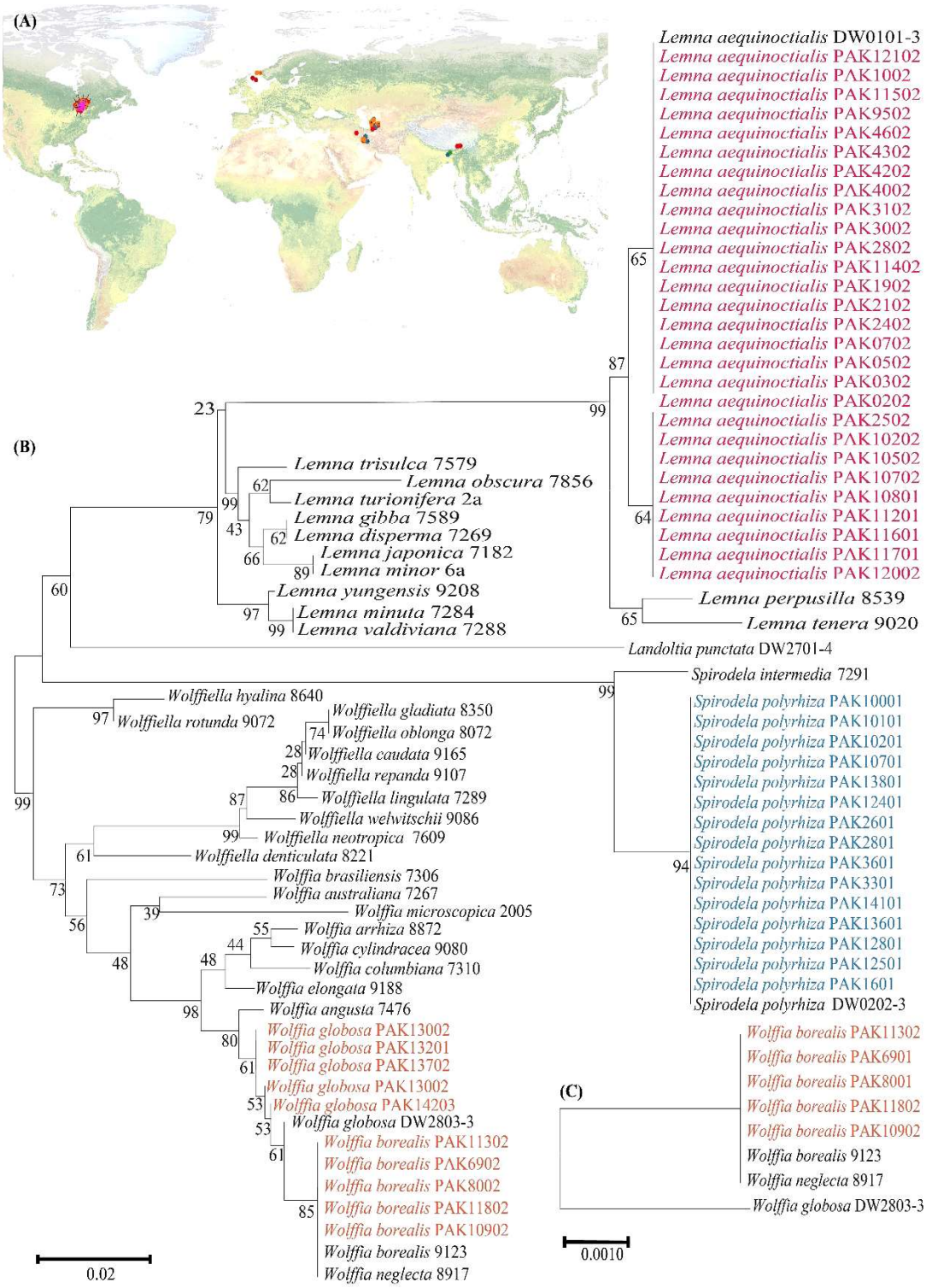

Fig. 5: Phylogenetic analysis based on *atpF-atpH* sequences. (A) Map of sampling localities of strains used in this phylogenetic analysis. (B) A Neighbor-Joining (NJ) tree constructed using the chloroplast *atpF-atpH* gene from duckweed. (C) Enlarged subtree from the main phylogenetic tree showing detailed relationships among *W. borealis* accessions. Positions with gaps or missing data were excluded by complete deletion, leaving a final dataset of 374 positions. Evolutionary analyses were performed with MEGA11, and the scale bar corresponds to 0.02 substitutions per site.

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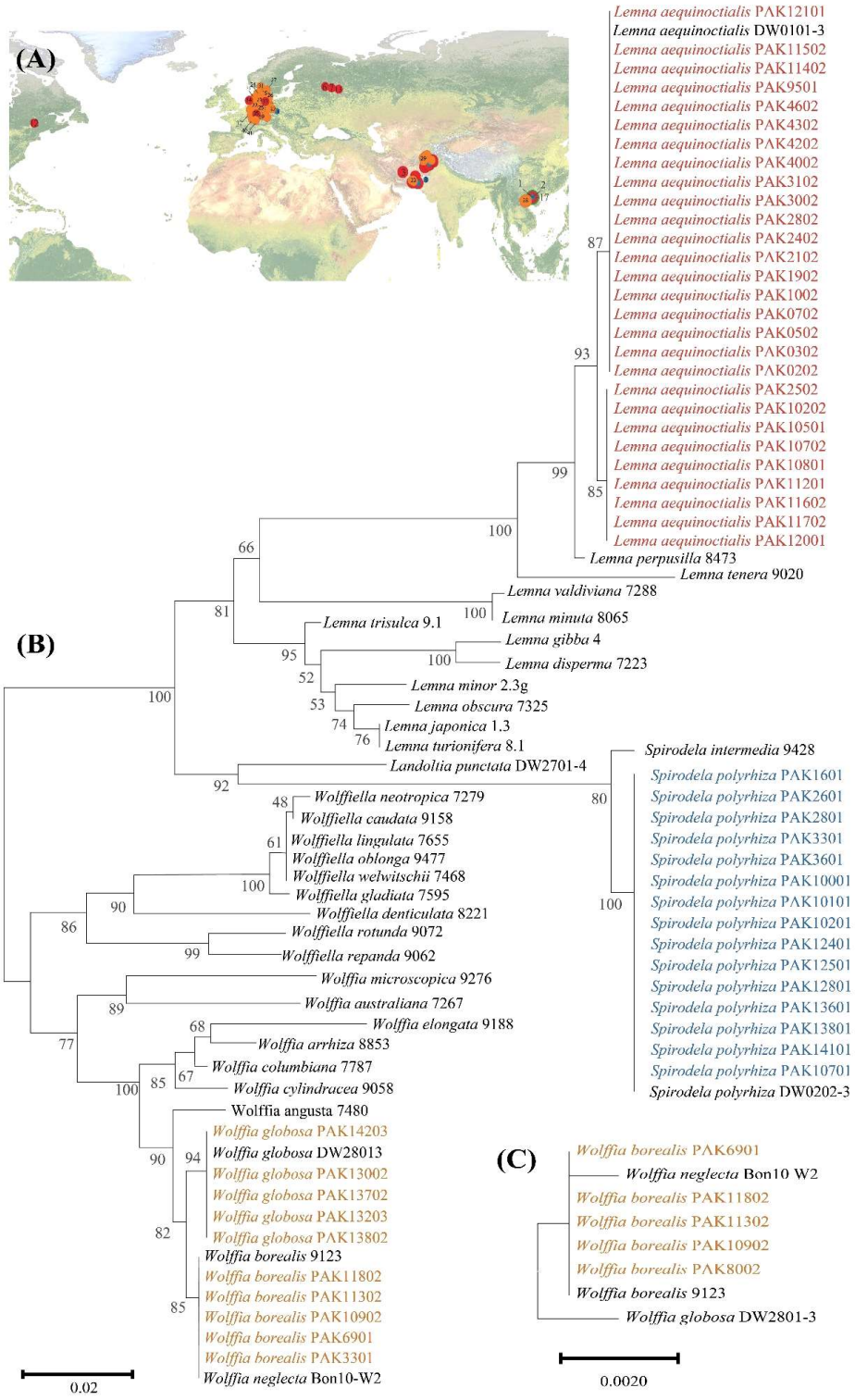

Fig. 6: Phylogenetic analysis based on *rps16* sequences. (A) Map of sampling localities used in this phylogenetic analysis. (B) A Neighbor-Joining (NJ) tree was constructed using the chloroplast *rps16* gene from duckweed. (C) Enlarged subtree from the main phylogenetic tree showing detailed relationships among *W. borealis* accessions Positions with gaps or missing data were excluded by complete deletion, leaving a final dataset of 491 positions. Evolutionary analyses were performed with MEGA11, and the scale bar corresponds to 0.02 substitutions per site.

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aequinoctialis populations and the homogeneity of other taxa signals the importance of species- and region-specific analyses in biodiversity assessments.

The patterns identified here reflect broader global observations in duckweed research. Like our findings, *L. aequinoctialis* has shown high intraspecific variation in East Asia (Xu et al., 2015), while *S. polyrhiza* consistently displaysminimal plastid diversity, likely due to its predominantly clonal reproduction and historical bottlenecks (Wang & Messing, 2015; Xu et al., 2015). The low variability observed in Wolffia species here aligns with earlier reports attributing this uniformity to strict vegetative propagation and limited dispersal potential (Bog et al., 2019). Our confirmation that *rps16* provides greater resolution than *atpF–atpH* for species and population-level differentiation reinforces its value as a primary barcode marker (He et al., 2025; Tippery & Les, 2020). Furthermore, the elevated diversity in southeastern populations of *L. aequinoctialis* supports ecological theories that environmental heterogeneity drives genetic diversification in aquatic macrophytes (Appenroth et al., 2017; Ekperusi et al., 2019; Anjur et al., 2024; Rodriguez et al., 2025). However, limitations must be acknowledged. Chloroplast DNA, while informative, evolves slowly and does not capture biparental or recombination-based variation. Under-sampling in some taxa and regions may obscure rare alleles, and the clonal nature of duckweeds may mask hidden diversity unless nuclear or genome-wide data are employed. Therefore, caution is warranted when interpreting "genetic uniformity, particularly in vegetatively reproducing species.

Despite methodological constraints, this study provides foundational insight into the evolutionary biology and conservation genetics of duckweeds in Pakistan. The identification of genetically diverse *L. aequinoctialis* populations, especially in the southeast, presents valuable opportunities for biotechnology. These populations may harbor traits critical for phytoremediation, biofuel generation, or nutritional enhancement, aligning with previous research demonstrating duckweeds′ roles in wastewater treatment (Zhao et al., 2014), starch-rich biomass production under nitrogen limitation (Guo et al., 2020), and sustainable protein alternatives (Sosa et al., 2024). In contrast, the uniformity in *S. polyrhiza* and *Wolffia* may signal lower adaptability under climate or anthropogenic pressures, a trend mirrored in genomics studies showing streamlined gene families in *S. polyrhiza* (Wang & Messing, 2015). Conservation-wise, the findings emphasize the need to prioritize genetically rich populations for protection, particularly in aquatic ecosystems facing habitat degradation and climate vulnerability.

Genetic diversity analyses revealed clear differences among the four duckweed species identified in this study. *L. aequinoctialis* exhibited the highest levels of chloroplast genetic diversity across sampling regions, whereas *S. polyrhiza* showed comparatively low variation despite its wide geographic distribution. Both *W. globosa* and *W. borealis* displayed very limited genetic diversity across the analysed markers. These patterns were consistent across multiple diversity indices and geographic groupings, indicating that species-level differences in genetic structure are a prominent feature of duckweed populations in Pakistan.

The contrasting patterns of genetic diversity observed among duckweed species in Pakistan are likely driven by differences in reproductive biology, dispersal ability, and ecological tolerance. *L. aequinoctialis* exhibits predominantly clonal propagation combined with occasional sexual reproduction, a strategy that can promote the accumulation of chloroplast haplotypes across heterogeneous environments. Its broad ecological distribution, including canals, agricultural waters, floodplain ponds, and wetlands, likely facilitates population subdivision and local adaptation, particularly in the climatically diverse southeastern regions of Pakistan. In contrast, *S. polyrhiza*, *W. borealis*, and *W. globosa* reproduce almost exclusively vegetatively and often form rapidly expanding clonal populations, which can reduce effective population size and constrain plastid genetic diversity. Historical population bottlenecks, restricted dispersal, and low plastid mutation rates may further contribute to the genetic uniformity observed in *S. polyrhiza*, *W. borealis*, and *W. globosa*. Together, these species-specific life-history traits provide a plausible explanation for the substantially higher chloroplast diversity observed in *L. aequinoctialis* compared with other duckweed species in Pakistan.

Finally, this work opens critical avenues for future research: Are nuclear genomes equally invariant in *S. polyrhiza, W. globosa* and *W. borealis*? What selective pressures contribute to southeastern *L. aequinoctialis* divergence? Could hybridization or introgression be shaping cryptic diversity in *Lemnaceae*? Addressing these questions with genome-wide approaches and expanded geographic sampling is essential to fully understand duckweed evolution and its applied potential. In essence, this study integrates local molecular data into the global narrative of duckweed biodiversity, offering a strategic framework for both basic and applied plant science.

5. CONCLUSION

This study presents the first comprehensive assessment of duckweed diversity and chloroplast genetic variation across Pakistan. By integrating extensive field sampling, axenic culture establishment, morphological identification, and chloroplast DNA barcoding, we demonstrate that *L. aequinoctialis* exhibits substantial genetic diversity, whereas *S. polyrhiza*, *W. borealis* and *W. globosa* show pronounced genetic uniformity at the chloroplast loci examined.

The contrasting patterns of diversity observed among species highlight the influence of species-specific life-

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history traits, ecological breadth, and geographic context on duckweed population structure. In particular, the elevated genetic variation detected in southeastern populations of *L. aequinoctialis* suggests greater evolutionary potential and local adaptation in this region. Phylogenetic analyses further confirm that the *rps16* intron provides greater resolution than *atpF–atpH*, supporting its use as a robust primary barcode marker for duckweed species identification and population-level studies.

Beyond documenting species presence, this work establishes a verified collection of axenic duckweed strains and provides foundational genetic data for an understudied region of South Asia. These resources will facilitate future investigations into duckweed evolution, conservation, and applied uses in biotechnology, including phytoremediation, sustainable biomass production, and alternative protein sources. Together, our findings contribute to global *knowledge of Lemnaceae biodiversity and provide a framework for expanded genomic and ecological studies of duckweeds across* climatically diverse regions.

In conclusion, this study provides the first integrative molecular and taxonomic assessment of duckweed populations in Pakistan, demonstrating that the combined use of complementary chloroplast markers enhances species identification and reveals contrasting pattern of genetic diversity among taxa. These finding have clear implications for conservation, ecological monitoring, and future biotechnological applications. The molecular framework established here offers a foundation for expanded genomic studies and contributes to a deeper understanding of duckweed evolution, adaption and utility in sustainable agriculture and environmental remediation.

Declarations

Funding: This research was partly supported by the Central Public-interest Scientific Institution Basal Research Fund (NO. 1630052025008).

Conflicts of Interest: The authors declare no competing interest.

Data Availability: Data will be made available on request.

Ethics Statement: This study involved only plant materials (duckweeds, family Lemnaceae) collected from natural freshwater habitats. No experiments involving human participants or animals were conducted. Therefore, ethical approval was not required. All sample collection and experimental procedures were carried out in accordance with relevant institutional guidelines and local regulations.

Author's Contributions: Umar Imtiaz: Sample collection, Investigation, Formal analysis, Original draft, revision. Khubaib Shakoor: Sample collection, Manuscript revision. Xiangshe Wang: Review, Validation, Project. Kede Liu: Methodology, Conceptualization. Deguan Tan: Formal analysis, Data curation, Revision. Jiaming Zhang: Writing – review and editing, Conceptualization, Supervision, Funding acquisition.

Generative AI Statements: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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REFERENCES

Ali, M. A., Gyulai, G., Hidvegi, N., Kerti, B., Al Hemaid, F. M., Pandey, A. K., & Lee, J. (2014). The changing epitome of species identification–DNA barcoding. Saudi Journal of Biological Sciences, 21(3), 204-231. https://doi.org/10.1016/j.sjbs.2014.03.003

Anjur, N., Ali, J., & Shahadan, S. A. (2024). Effect of different fertilizers on the growth of duckweed (Lemnaminor) as aquatic plant resources utilization in sustaining Red Tilapia (Oreochromis niloticus) culture. In E3S Web of Conferences (Vol. 479, p. 03001). EDP Sciences. https://doi.org/10.1051/e3sconf/202447903001

Appenroth, K.-J., Sree, K. S., Böhm, V., Hammann, S., Vetter, W., Leiterer, M., & Jahreis, G. (2017). Nutritional value of duckweeds (Lemnaceae) as human food. Food Chemistry, 217, 266-273. https://doi.org/10.1016/j.foodchem.2016.08.116

Asniarti, A., Fahruddin, F., & Tambaru, E. (2025). Phytoremediation of ammonia and water quality improvement in common carp (Cyprinus carpio) culture using Ipomoea aquatica and Lemnaminor. Ecological Engineering & Environmental Technology, 26(10), 343-355. https://doi.org/10.12912/27197050/210714

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Open Access Journal

Baek, G., Saeed, M., & Choi, H.-K. (2021). Duckweeds: their utilization, metabolites and cultivation. Applied Biological Chemistry, 64(1), 73. https://doi.org/10.1186/s13765-021-00644-z

Bog, M., Appenroth, K.-J., & Sree, K. S. (2019). Duckweed (Lemnaceae): its molecular taxonomy. Frontiers in Sustainable Food Systems, 3, 117. https://doi.org/10.3389/fsufs.2019.00117

Bog, M., Schneider, P., Hellwig, F., Sachse, S., Kochieva, E. Z., Martyrosian, E., Landolt, E., & Appenroth, K.-J. (2013). Genetic characterization and barcoding of taxa in the genus Wolffia Horkel ex Schleid.(Lemnaceae) as revealed by two plastidic markers and amplified fragment length polymorphism (AFLP). Planta, 237(1), 1-13. https://doi.org/10.1007/s00425-012-1777-9

Ekperusi, A. O., Sikoki, F. D., & Nwachukwu, E. O. (2019). Application of common duckweed (Lemnaminor) in phytoremediation of chemicals in the environment: State and future perspective. Chemosphere, 223, 285-309. https://doi.org/10.1016/j.chemosphere.2019.02.025

Escobar, C., & Escobar, A. (2017). Duckweed: A tiny aquatic plant with enormous potential for bioregenerative life support systems 47th International Conference on Environmental Systems, Charleston, SC, USA.

Faizal, A., Sembada, A. A., & Priharto, N. (2021). Production of bioethanol from four species of duckweeds (Landoltia punctata, Lemna aequinoctialis, Spirodela polyrrhiza, and Wolffia arrhiza) through optimization of saccharification process and fermentation with Saccharomyces cerevisiae. Saudi Journal of Biological Sciences 28(1), 294-301. https://doi.org/10.1016/j.sjbs.2020.10.002

Fu, L., Ding, Z., Tan, D., Han, B., Sun, X., & Zhang, J. (2020). Genome-wide discovery and functional prediction of salt-responsive lncRNAs in duckweed. BMC Genomics, 21(1), 212. https://doi.org/10.1186/s12864-020-6633-x

Fu, L., Huang, M., Han, B., Sun, X., Sree, K. S., Appenroth, K.-J., & Zhang, J. (2017). Flower induction, microscope-aided cross-pollination, and seed production in the duckweed Lemna gibba with discovery of a male-sterile clone. Scientific Reports, SREP-17-01786, DOI : https://doi.org/01710.01038/s41598-01017-03240-01788

Guo, L., Jin, Y., Xiao, Y., Tan, L., Tian, X., Ding, Y., He, K., Du, A., Li, J., & Yi, Z. (2020). Energy-efficient and environmentally friendly production of starch-rich duckweed biomass using nitrogen-limited cultivation. Journal of Cleaner Production, 251, 119726. https://doi.org/10.1016/j.jclepro.2019.119726

Han, B., Chen, T., Yu, B., Ren, Y., Long, Y., Long, Y., Tan, D., Fu, L., & Zhang, J. (2022). Nutritional value of domesticated duckweed variety DW2602 and its feeding effects on the growth performance and digestive activities of tilapia fingerlings. Tropical Plants, 1(11), 1-9. https://doi.org/10.48130/TP-2022-0011

He, X., Yang, Y., Zhang, X., Zhao, W., Zhang, Q., Luo, C., Xie, Y., Li, Z., & Wang, X. (2025). Comparative chloroplast genomics of Actinidia deliciosa cultivars: Insights into positive selection and population evolution. International Journal of Molecular Sciences, 26(9), 4387. https://doi.org/10.3390/ijms26094387

Iqbal, J., Javed, A., & Baig, M. A. (2019). Growth and nutrient removal efficiency of duckweed (Lemnaminor) from synthetic and dumpsite leachate under artificial and natural conditions. PloS one, 14(8), e0221755. https://doi.org/10.1371/journal.pone.0221755

Jaimes Prada, O., Lora Díaz, O., & Tache Rocha, K. (2024). Common duckweed (Lemnaminor): food and environmental potential. Review. Revista Mexicana de Ciencias Pecuarias, 15(2), 404-424. https://doi.org/10.22319/rmcp.v15i2.6107

Kafle, A., Timilsina, A., Gautam, A., Adhikari, K., Bhattarai, A., & Aryal, N. (2022). Phytoremediation: Mechanisms, plant selection and enhancement by natural and synthetic agents. Environmental Advances, 8, 100203. https://doi.org/10.1016/j.envadv.2022.100203

Lee, C. J., Yangcheng, H., Cheng, J. J., & Jane, J. l. (2016). Starch characterization and ethanol production of duckweed and corn kernel. Starch-Stärke, 68(3-4), 348-354. https://doi.org/10.1002/star.201500126

Mustafa, H. M., & Hayder, G. (2021). Recent studies on applications of aquatic weed plants in phytoremediation of wastewater: A review article. Ain Shams Engineering Journal, 12(1), 355-365. https://doi.org/10.1016/j.asej.2020.05.009

Prada, O. J., Díaz, O. L., & Rocha, K. T. (2024). Common duckweed (Lemnaminor): Food and environmental potential. Review. Rev. Mex. Cienc. Pecu, 15, 404-424. https://doi.org/10.22319/rmcp.v15i2.6107

Rodriguez, J. H. V., Gavin-Moyano, C., Aveiga, M. D. R. V., Mata, J. D. O., Carrasco, J. D. S., Vidal, L. R. L., & Mata, B. E. G. (2025). Chemical study of the macrophyte duckweed (Lemnaminor L.). Revista De La Facultad De Agronomia De La Universidad Del Zulia, 42(1), e254202. https://doi.org/10.47280/RevFacAgron(LUZ).v42.n1.II

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution, 34(12), 3299-3302. https://doi.org/10.1093/molbev/msx248

Sońta, M., Rekiel, A., & Batorska, M. (2019). Use of duckweed (Lemna L.) in sustainable livestock production and aquaculture–a review. Annals of Animal Science, 19(2), 257-271. https://doi.org/10.2478/aoas-2018-0048

Sosa, D., Alves, F. M., Prieto, M. A., Pedrosa, M. C., Heleno, S. A., Barros, L., Feliciano, M., & Carocho, M. (2024). Lemnaminor: Unlocking the value of this duckweed for the food and feed industry. Foods, 13(10), 1435. https://doi.org/10.3390/foods13101435

Sree, K. S., Bog, M., & Appenroth, K.-J. (2016). Taxonomy of duckweeds (Lemnaceae), potential new crop plants. Emirates Journal of Food & Agriculture (EJFA), 28(5). https://doi.org/10.9755/ejfa.2016-01-038

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution, 10(3), 512-526. https://doi.org/10.1093/oxfordjournals.molbev.a040023

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution, 38(7), 3022-3027. https://doi.org/10.1093/molbev/msab120

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

Thompson, J. D., Gibson, T. J., & Higgins, D. G. (2003). Multiple sequence alignment using ClustalW and ClustalX. Current Protocols in Bioinformatics (1), 2.3. 1-2.3. 22. https://doi.org/10.1002/0471250953.bi0203s00

Tippery, N. P., & Les, D. H. (2020). Tiny plants with enormous potential: phylogeny and evolution of duckweeds. In The Duckweed Genomes (pp. 19-38). Springer. https://doi.org/10.1007/978-3-030-11045-1_2

Ullah, H., Gul, B., Khan, H., & Zeb, U. (2021). Effect of salt stress on proximate composition of duckweed (Lemnaminor L.). Heliyon, 7(6). https://doi.org/10.1016/j.heliyon.2021.e07399

Walsh, É., Coughlan, N. E., O'Brien, S., Jansen, M. A., & Kuehnhold, H. (2021a). Density dependence influences the efficacy of wastewater remediation by Lemnaminor. Plants, 10(7), 1366. https://doi.org/10.3390/plants10071366

Walsh, É., Kuehnhold, H., O'Brien, S., Coughlan, N. E., & Jansen, M. A. (2021b). Light intensity alters the phytoremediation potential of Lemnaminor. Environmental Science and Pollution Research, 28, 16394-16407. https://doi.org/10.1007/s11356-020-11-792-y

Walsh, É., Paolacci, S., Burnell, G., & Jansen, M. A. (2020). The importance of the calcium-to-magnesium ratio for phytoremediation of dairy industry wastewater using the aquatic plant Lemnaminor L. International Journal of Phytoremediation, 22(7), 694-702. https://doi.org/10.1080/15226514.2019.1707478

Wang, W., & Messing, J. (2015). Status of duckweed genomics and transcriptomics. Plant Biology, 17, 10-15. https://doi.org/10.1111/plb.12201

Xu, J., Shen, Y., Zheng, Y., Smith, G., Sun, X. S., Wang, D., Zhao, Y., Zhang, W., & Li, Y. (2023). Duckweed (Lemnaceae) for potentially nutritious human food: A review. Food Reviews International, 39(7), 3620-3634. https://doi.org/10.1080/87559129.2021.2012800

Xu, Y., Ma, S., Huang, M., Peng, M., Bog, M., Sree, K. S., Appenroth, K.-J., & Zhang, J. (2015). Species distribution, genetic diversity and barcoding in the duckweed family (Lemnaceae). Hydrobiologia, 743, 75-87. https://doi.org/10.1007/s10750-014-2014-2

Zhao, Y., Fang, Y., Jin, Y., Huang, J., Bao, S., Fu, T., He, Z., Wang, F., & Zhao, H. (2014). Potential of duckweed in the conversion of wastewater nutrients to valuable biomass: a pilot-scale comparison with water hyacinth. Bioresource Technology, 163, 82-91. https://doi.org/10.1016/j.biortech.2014.04.018

Zhou, Y., & Borisjuk, N. (2019). Small aquatic duckweed plants with big potential for the production of valuable biomass and wastewater remediation. International Journal Environmental Science Natural Resources, 16, 555942. https://doi.org/10.19080/IJESNR.2019.16.555942