

# Morphological and Molecular Characterization of Moss Species Widespread in Syria

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## Abstract

This study addresses a significant knowledge gap in Syria's bryophyte diversity by conducting an integrated analysis of moss species from two climatically distinct regions: the drier inland area of Al-Zabadani and the more humid coastal region of Al-Haffah. By combining morphological examination with molecular techniques, we characterized 16 Bryopsida species using 12 morphological parameters and the RAPD method with 10 primers. Our results clearly show that the humid conditions of Al-Haffah support a greater diversity of mosses compared to the drier Al-Zabadani region. While morphological analysis successfully grouped the species into four clusters at the order level, the molecular data provided a higher resolution, distinguishing five distinct groups at the family level and revealing a high degree of genetic polymorphism (701 bands, PIC values 0.28-0.37). Methodologically, we successfully extracted high-purity DNA using the CTAB method and found the RAPD technique to be highly effective, with primer OP-R11 performing particularly well (25 markers and 120 bands). By establishing the first detailed moss database for these Syrian regions and validating a combined morphological-molecular framework, this study provides essential foundations for advancing bryophyte conservation and ecological research in similar Mediterranean environments.

**Keywords:** Bryopsida, acrocarpus, pleurocarpus, RAPD, cluster analysis, Syria.

## 1. Introduction

Bryophytes are important worldwide plant groups, they constitute the second most diverse group of land plants after flowering plants. Taxonomically, Bryophytes are situated between the algae and the pteridophytes. Their life cycle includes two generations: a gametophyte (haploid) and a sporophyte (diploid) stages. They are morphologically divided into three phyla: Marchantiophyta (liverworts), Anthocerotophyta (hornworts) and Bryophyta (mosses). They are small plants that grow on various types of substrates such as moist soil, tree trunks and branches, and rocks. They can be found in all ecosystems except marine environments; however, they prefer moist and shady habitats (Dziwak *et al.*, 2022; Dhyani *et al.*, 2025). In Syria, it is widespread throughout the western part, from the Turkish border in the north to Al-Golan in the south, including Syrian coastal strip forests and mountainous regions (Ismaeel *et al.*, 2024a).

Bryophytes hold significant value in both traditional and modern medicine. Historically used across various cultures for treating ailments like skin wounds and liver disorders, their medicinal potential was first documented in 1957. Contemporary research confirms their role as sources of antibiotics, antioxidants, and potential anti-cancer agents (Mandić *et al.*, 2021; Motti *et al.*, 2023; Ismaeel *et al.*, 2024b). Furthermore, they have emerged as

promising biopharmaceutical platforms, with *Physcomitrella patens* being the first plant to produce human proteins " such as vascular endothelial growth factor (VEGF) and alpha-galactosidase ( $\alpha$ -Gal)", positioning bryophytes as potential candidates for vaccine production (Reski *et al.*, 2015).

Beyond their medicinal value, bryophytes serve important economic and ecological roles. Economically, they are utilized as heating fuel, food, and animal feed (Klavina, 2015; Ismaeel *et al.*, 2022). Ecologically, they significantly contribute to humid environment biodiversity through their exceptional water retention capacity and play crucial roles in nutrient cycling, particularly for carbon and nitrogen (Condes *et al.*, 2023). Furthermore, their effectiveness as bioindicators for pollution monitoring has been well documented (Ismaeel, 2017; Nurkassimova *et al.*, 2024).

While morphological criteria have traditionally served as the primary basis for plant classification, the growing need for precise species identification to support conservation efforts has driven the adoption of molecular techniques in recent decades. Molecular markers such as RAPD, ISSR, and SSR have become pivotal tools in phylogenetic and taxonomic studies (Crespo Pardo *et al.*, 2014; Dhyani *et al.*, 2025). These molecular markers "defined as heritable DNA sequences at specific genomic loci" provide high resolution data that elucidating taxonomic relationships between genera and species, often obscured by morphological analysis alone (Vanderpoorten

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and Jacquemart, 2004; Lang *et al.*, 2015; Schlesak *et al.*, 2018).

Among these methods, Random Amplified Polymorphic DNA (RAPD) has proven particularly valuable in bryological research since its introduction in the 1990s. Despite its limitations, including band irreproducibility under low stringency conditions, dominant inheritance patterns, uncertain amplification products, and co-migration of non-homologous fragments of similar size, the RAPD technique remains highly suitable for moss studies. This suitability stems from the small, often unsequenced genomes and haploid nature of mosses, which avoids complications of dominant marker expression common in diploid organisms (Arif *et al.*, 2010; Crespo Pardo *et al.*, 2014). By amplifying random DNA fragments using short primers, RAPD enables genetic diversity assessment without prior sequence knowledge and requires minimal sample material (Dale *et al.*, 1999).

The field of bryophyte molecular research has expanded significantly since the pioneering DNA sequencing study in 1983, accumulating approximately 382 studies by 2009 (Stech and Quandt, 2010). The RAPD technique has been widely applied to elucidate intraspecific genetic variation across diverse ecological contexts. Initial investigations on *Henediella heimii* demonstrated population level genetic differentiation (Dale *et al.*, 1999). Findings later extended to related mosses species, including *Bryum argenteum*, *B. pseudotriquetrum*,

and *Ceratodon purpureus* from Antarctic environments (Selkirk *et al.*, 1998; Skotnicki *et al.*, 1998; Clarke *et al.*, 2009). Collectively, these studies establish RAPD as an effective methodology for resolving genetic relationships among bryophytes.

In response to the scarcity of data on Syrian bryophytes, which has historically been confined to morphological descriptions, this study provides a taxonomic and genetic survey of moss species from the ecologically contrasting regions of Al-Zabadani and Al-Haffah. The study involves identification using bryological taxonomic keys, complemented by a molecular analysis using RAPD markers to determine interspecific genetic relationships. The morphological documentation will subsequently be compared with cluster analysis results from the genetic data to establish a unified classification.

## 2. Materials and methods

### 2.1. Sample Collection

Mosses samples were collected from Al-Zabadani "Damascus countryside" (33°06'62"N 36°08'66"E) and Al-Haffah "Latakia" (35°05'58"N 36°05'72"E) regions in Syria during multiple field trips between January and April 2023 (Figure 1), coinciding with the annual growth and spread gametophyte generations of mosses associated with suitable precipitation and temperature conditions. Samples were then transferred to the laboratory and stored.



**Figure 1.** General location of the studied areas (Al-Zabadani and Al-Haffah regions).

### 2.2. Morphological and taxonomic study

Morphological analyses were conducted at the Plant Biology Department, University of Damascus. Species identification was performed using light macroscopy and microscopy, with taxonomic classification based on morphological and anatomical characteristics commonly used in bryophytes classification, including leaf and costa length, leaf margins, capsule shape, stem branching, and more. The observed features were systematically compared with standard bryological taxonomic keys and literature (Kurschner, 2006, 2007, 2008; Goffinet and

Shaw, 2008). Twelve morphological parameters were evaluated using SPSS V22 (fifty replicates each). The quantified characteristics encompassed sporophyte dimensions (sporophyte plant, seta, and capsule length), leaf length, costa length, costa width, and cellular characteristics (basal, lamina, and apical cell measurements, including length, and length-to-width ratios), as these morphological criteria are commonly used in classification.

### 2.3. Molecular study (Molecular Characterization)

The molecular study was conducted to investigate the genetic relationships and species clustering. Molecular analysis was carried out in the Graduate Studies Laboratory (Department of Plant Biology, Faculty of Science, Damascus University), using RAPD technique, as follows:

#### 2.3.1. DNA extraction

Samples were thoroughly cleaned with distilled water, and old parts (brown parts) were removed. DNA extraction was performed using CTAB method, as described by Skotnicki *et al.* (2005), with minor modifications. A freshly prepared 1% CTAB extraction solution was used, prepared as follows: 2.5 mL of 1M Tris-HCl, 2.5 mL of 0.5M EDTA, 3.5 mL of 5M NaCl, 250 mg of CTAB, 250 mg of PVP, and 25  $\mu$ L of  $\beta$ -mercaptoethanol, with the final volume adjusted to 25 mL using distilled water (Table 1).

**Table 1.** Composition and final concentrations of the 1% CTAB extraction buffer.

Material	Volume/Weight for 25 mL	Final concentrations
Tris-HCl (1M) - pH:7.5	2.5 mL	100 mM
EDTA (0.5 M) - pH: 8	2.5 mL	50 mM
NaCl (5M)	3.5 mL	1.4 M
CTAB	250 mg	1%
PVP	250 mg	1%
$\beta$ -mercaptoethanol	25 $\mu$ L	0.1%
Distilled water	Add to 25 mL	

For acrocarpous species, DNA was extracted using single shoots to avoid contamination from species overlap within moss mats, with each species' individual placed in a separate tube (Figure 2). For pleurocarpous species, where there is no risk of interspecies interference, DNA extraction was performed using 100 mg of material per species.



**Figure 2.** Samples of single shoots of some acrocarpous species in Eppendorf tubes.

#### 2.3.2. Quantitative and Purity Analysis of DNA

DNA concentration and purity were assessed using a UV-Visible spectrophotometer (Mecasys - Korea). Absorbance was measured at 260 nm ( $OD_{260}$ ) and 280 nm ( $OD_{280}$ ), with concentration calculated according to the standard formula (Besse, 2014): DNA Concentration ( $\mu$ g/mL) =  $OD_{260} \times 50 \times$  Dilution Factor, while DNA purity was evaluated using the  $OD_{260}/OD_{280}$  ratio, with values

between 1.8 and 2.0 indicating high purity DNA. To verify these measurements, DNA concentration was additionally quantified using a NanoDrop 2000 spectrophotometer "Thermo Fisher Scientific" (Shen *et al.*, 2022).

#### 2.3.3. Amplification Using RAPD Technique

The Random Amplified Polymorphic DNA (RAPD) technique was employed for molecular characterization, genetic distance estimation among collected moss species, as well as for cluster analysis. DNA amplification was performed using ten randomly selected primers from Alpha DNA (Operon), as detailed in Table 2.

**Table 2.** RAPD Primers used for Polymerase Chain Reaction (PCR) amplification:

Primer Number	Primer Code	Sequences (5'-3')	Annealing Temperature ( $^{\circ}$ C)
1	OP-Z14	TCGGAGGTTC	32
2	OP-R15	GGACAACGAG	32
3	OP-R11	GTAGCCGTCT	32
4	OP-R07	ACTGGCCTGA	32
5	OP-E12	TTATCGCCCC	32
6	OP-E01	CCCAAGGTCC	34
7	OP-E18	GGACTGCAGA	32
8	OP-Z13	GACTAAGCCC	32
9	OP-R04	CCCGTAGCAC	34
10	OP-R08	CCCGTTGCCT	34

RAPD reactions were conducted using GoTaq® Green Master Mix (2X) (Promega, USA) in a 25  $\mu$ L final reaction volume, with modifications introduced to the manufacturer's protocol to optimize reaction conditions. Each reaction contained: 12.5  $\mu$ L master mix, 2  $\mu$ L DNA template (approximately 100 ng  $\mu$ L<sup>-1</sup>), 1.5  $\mu$ L primer, 9  $\mu$ L distilled water. Reaction mixtures were overlaid with 40  $\mu$ L of sterile mineral oil to prevent evaporation during thermal cycling.

Amplification was conducted in a GenePro Thermocycler (BIOER, USA) programmed as follows:

- Initial denaturation: 94 $^{\circ}$ C for 3 minutes.
- Amplification: 45 cycles of:
  1. Denaturation: 94 $^{\circ}$ C for 1 minute.
  2. Primer annealing: 32-34  $^{\circ}$ C for 1 minute (primer specific).
  3. Elongation: 72 $^{\circ}$ C for 2 minutes.
- Final elongation: 72 $^{\circ}$ C for 10 minutes.

The annealing according temperature was optimized to the specific primers employed in this study (32 - 34  $^{\circ}$ C). It should be noted that annealing temperature varies depending on the nucleotide composition of individual primers.

#### 2.3.4. Electrophoresis of amplification products

Amplification products were separated on a 1.8% (w/v) agarose gel in 1X TBE buffer containing 5  $\mu$ L of ethidium bromide (10 mg/ml). Samples were electrophoresed at 100 volts for 2 hours (Electrophoresis Power Supply-Consort EV243) in TBE buffer. DNA bands were visualized by staining with ethidium bromide and photographed using a gel documentation system (Clever Scientific - UK) equipped with a UV filter. Additionally, 5  $\mu$ L of a 100 bp DNA ladder (GeneDirex - USA) was loaded onto the gel as a molecular weight standard. Following amplification,

the resulting DNA profiles were examined under UV light and photographed for analysis.

#### 2.4. Statistical study

Statistical analysis of the morphological parameters for the all species was performed using SPSS V22 software. Means were calculated, and differences between them were assessed for statistical significance using a One-Way ANOVA test, with a significance level of  $P < 0.05$  considered statistically significant at a 95% confidence level. Additionally, a dendrogram was constructed based on morphological criteria using SPSS V22 (fifty replicates each).

The Polymorphism Information Content (PIC) represents a fundamental metric for assessing a genetic marker's ability to detect polymorphisms, thereby serving as a critical tool in marker selection for genetic research (Kulibaba *et al.*, 2025). PIC is calculated using the established formula:  $PIC_i = 2f_i(1-f_i)$ , where  $PIC_i$  represents the degree of variation between species for molecular index  $i$ ,  $f_i$  represents the frequency of bands presence, while  $1-f_i$  corresponds to the frequency of band absence. A dendrogram was constructed using the Jaccard similarity coefficient with the paired groups algorithm (UPGMA) in Past Statistical Software Version 1.92. Our team manually scored only the clear and reproducible amplification bands, with multiple observers independently visually inspecting and cross-checking the results to minimize bias. Each band was manually recorded as present (1) or absent (0) without using automated scoring software. We then built a data matrix from these binary scores and performed a cluster analysis to construct a genetic similarity dendrogram (Mokrani and Al-Ashkar, 2022).

### 3. Results

#### 3.1. Climate study

Climate data from 2010 to 2022 indicated that the average relative humidity in Al-Haffah ranged from 62% to 76%, with a total precipitation of 998 mm, and a temperature average varying between 10°C and 27°C. The actual dry period during summer season lasted for three months. However, in Al-Zabadani, precipitation totaled approximately 662 mm, with an average temperature ranging from 4°C to 22°C. Relative humidity varied between 46% and 76%. The actual dry period during summer season extended to five months. These values are consistent with several local studies for both regions (Ismacel, 2017; Saeed and Hasso, 2021).

#### 3.2. Classification study

This study aims to complement previous research on mosses in Syria, with a specific focus on the Al-Zabadani (Damascus countryside Governorate) and Al-Haffah (Latakia Governorate) regions. A comprehensive morphological analysis was conducted for all collected specimens, with classification based on standardized morphological and anatomical criteria as detailed in the Materials and Methods. Taxonomic identification was verified through comparison with established taxonomic keys and relevant literature.

The investigation revealed that all identified species belong to the class Bryopsida, comprising sixteen distinct species: ten acrocarpous species and six pleurocarpous mosses. Distribution analysis showed twelve species in Al-Zabadani and eleven in Al-Haffah, with several species common to both regions (Tables 3-4).

Table 3. Categorization of species based on their distribution in the study areas.

Study areas	Species	No.
Al-Zabadani region-specific species	<i>Barbula unguiculata</i> Hedw.	1
	<i>Didymodon fallax</i> (Hedw.) R.H.Zander	2
	* <i>Amblystegium riparium</i> (Hedw.) Schimp.	3
	* <i>Hygroamblystegium tenax</i> (Hedw.) Jenn.	4
	* <i>Brachythecium erythrorrhizon</i> Schimp.	5
Species common to both regions	<i>Grimmia pulvinata</i> (Hedw.) Sm.	6
	<i>Schistidium apocarpum</i> (Hedw.) Bruch & Schimp.	7
	<i>Syntrichia ruralis</i> (Hedw.) F.Weber & D.Mohr	8
	<i>Bryum argenteum</i> Hedw.	9
	<i>Bryum caespiticium</i> Hedw.	10
	<i>Bryum capillare</i> Hedw.	11
	* <i>Eurhynchium praelongum</i> (Hedw.) Schimp.	12
Al-Haffah region-specific species	<i>Timmia anomala</i> (Bruch & Schimp.) Limpr.	13
	<i>Tortella fragilis</i> (Drumm.) Limpr.	14
	* <i>Leptodictyum riparium</i> (Hedw.) Warnst.	15
	* <i>Rhynchostegium murale</i> (Hedw.) Schimp.	16

The asterisk (\*) denotes pleurocarpous species.

**Table 4.** The species distributed in the two study regions.



*Didymodon fallax*



*Barbula unguiculata*



*Schistidium apocarpum*



*Grimmia pulvinata*



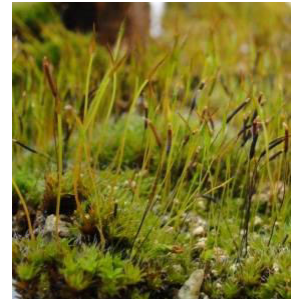
*Bryum argenteum*



*Tortella fragilis*



*Timmiella anomala*



*Syntrichia ruralis*



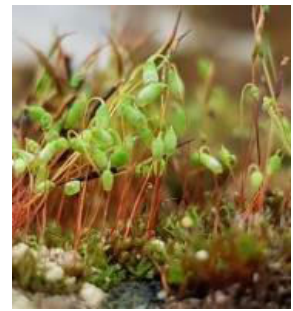
*Hygroamblystegium tenax*



*Amblystegium riparium*



*Bryum capillare*



*Bryum caespiticium*



*Rhynchostegium murale*



*Eurhynchium praelongum*



*Brachythecium erythrorrhizon*



*Leptodictyum riparium*

The species distribution between sites is presented below, where the equals symbol (=) indicates taxonomic synonyms:

- Species found in Al-Zabadani region
  1. *Grimmia pulvinata* (Hedw.) Sm.
  2. *Schistidium apocarpum* (Hedw.) Bruch & Schimp.
  3. *Barbula unguiculata* Hedw.
  4. *Didymodon fallax* (Hedw.) R.H.Zander  
= *Geheebia fallax* (Hedw.) R.H.Zander
  5. *Syntrichia ruralis* (Hedw.) F.Weber & D.Mohr
  6. *Bryum argenteum* Hedw.
  7. *Bryum caespiticium* Hedw.  
= *Ptychostomum imbricatum* (Müll.Hal.) Holyoak & N.Pedersen
  8. *Bryum capillare* Hedw.  
= *Rosulabryum capillare* (Hedw.) J.R.Spence
  9. *Amblystegium riparium* (Hedw.) Schimp.  
= *Leptodictyum riparium* (Hedw.) Warnst.
  10. *Hygroamblystegium tenax* (Hedw.) Jenn.
  11. *Brachythecium erythrorrhizon* Schimp.
  12. *Eurhynchium praelongum* (Hedw.) Schimp.  
= *Kindbergia praelonga* (Hedw.) Ochyra

Species found in Al-Haffah region

1. *Grimmia pulvinata* (Hedw.) Sm.
2. *Schistidium apocarpum* (Hedw.) Bruch & Schimp.
3. *Syntrichia ruralis* (Hedw.) F.Weber & D.Mohr
4. *Timmia anomala* (Bruch & Schimp.) Limpr.
5. *Tortella fragilis* (Drumm.) Limpr.
6. *Bryum argenteum* Hedw.
7. *Bryum caespiticium* Hedw.  
= *Ptychostomum imbricatum* (Müll.Hal.) Holyoak & N.Pedersen
8. *Bryum capillare* Hedw.  
= *Rosulabryum capillare* (Hedw.) J.R.Spence
9. *Leptodictyum riparium* (Hedw.) Warnst.
10. *Eurhynchium praelongum* (Hedw.) Schimp.  
= *Kindbergia praelonga* (Hedw.) Ochyra
11. *Rhynchostegium murale* (Hedw.) Schimp.

The statistical study was conducted using morphological criteria with 12 parameters and 50 replicates (as previously mentioned), employing One-way ANOVA at a significance level of  $P < 0.05$ , indicating statistical significance at a 95% confidence level. Our statistical analysis revealed clear morphological divergence between pleurocarpous and acrocarpous species (see table 5 for pleurocarpous; tables 6-7 for acrocarpous), underscoring the distinct phenotypic profiles that characterize these two growth forms.

**Table 5.** Statistical study of morphological criteria for pleurocarpous species.

Species	S.P.L/ mm	S.L/ mm	C.L/ mm	L.L/ mm	CO.L/ mm	CO.W/ $\mu\text{m}$
<i>Hygro. tenax</i> -Z	16.5 $\pm$ 0.71 <sup>a</sup>	14.5 $\pm$ 0.72 <sup>a</sup>	2 $\pm$ 0.14 <sup>a</sup>	1.85 $\pm$ 0.1 <sup>a</sup>	1.5 $\pm$ 0.11 <sup>a</sup>	78 $\pm$ 4.89 <sup>a</sup>
<i>A. riparium</i> -Z	20.7 $\pm$ 1.61 <sup>b</sup>	18.2 $\pm$ 1.59 <sup>b</sup>	2.4 $\pm$ 0.11 <sup>b</sup>	2.16 $\pm$ 0.11 <sup>b</sup>	1.3 $\pm$ 0.1 <sup>b</sup>	46 $\pm$ 4.88 <sup>b</sup>
<i>L. riparium</i> -L	17.3 $\pm$ 1.26 <sup>c</sup>	15.8 $\pm$ 1.24 <sup>c</sup>	1.5 $\pm$ 0.12 <sup>c</sup>	1.42 $\pm$ 0.05 <sup>c</sup>	1.1 $\pm$ 0.08 <sup>c</sup>	47 $\pm$ 7.23 <sup>b</sup>
<i>B. erythrorrhizon</i> -Z	13.6 $\pm$ 0.8 <sup>d</sup>	11.5 $\pm$ 0.83 <sup>d</sup>	2.1 $\pm$ 0.18 <sup>d</sup>	2.43 $\pm$ 0.18 <sup>d</sup>	1.2 $\pm$ 0.11 <sup>d</sup>	48 $\pm$ 4.77 <sup>b</sup>
<i>R. murale</i> -L	20.1 $\pm$ 1.7 <sup>e</sup>	17.8 $\pm$ 1.72 <sup>e</sup>	2.4 $\pm$ 0.15 <sup>e</sup>	1.60 $\pm$ 0.08 <sup>e</sup>	1.2 $\pm$ 0.1 <sup>b</sup>	56 $\pm$ 4.99 <sup>c</sup>
<i>E. praelongum</i> -Z	14.6 $\pm$ 1.52 <sup>f</sup>	12.7 $\pm$ 1.51 <sup>f</sup>	1.9 $\pm$ 0.12 <sup>f</sup>	1.15 $\pm$ 0.09 <sup>f</sup>	1.1 $\pm$ 0.12 <sup>c</sup>	47 $\pm$ 5.71 <sup>b</sup>
<i>E. praelongum</i> -L	12.1 $\pm$ 0.73 <sup>g</sup>	10.4 $\pm$ 0.74 <sup>g</sup>	1.8 $\pm$ 0.1 <sup>g</sup>	1.34 $\pm$ 0.06 <sup>g</sup>	1 $\pm$ 0.06 <sup>c</sup>	52 $\pm$ 4.06 <sup>d</sup>
Species	B.C.L/ $\mu\text{m}$	B.C.L/B.C.W	L.C.L/ $\mu\text{m}$	L.C.L/L.C.W	A.C.L/ $\mu\text{m}$	A.C.L/A.C.W
<i>Hygro. tenax</i> -Z	39.6 $\pm$ 3.8 <sup>a</sup>	7.14 $\pm$ 1.4 <sup>a</sup>	72.3 $\pm$ 3.72 <sup>a</sup>	13.4 $\pm$ 1.94 <sup>a</sup>	56.4 $\pm$ 5 <sup>a</sup>	9.3 $\pm$ 2.01 <sup>a</sup>
<i>A. riparium</i> -Z	69 $\pm$ 5.08 <sup>b</sup>	6 $\pm$ 0.94 <sup>b</sup>	91.7 $\pm$ 4.33 <sup>b</sup>	11.7 $\pm$ 1.42 <sup>b</sup>	71.6 $\pm$ 5.58 <sup>b</sup>	9.5 $\pm$ 2.86 <sup>ab</sup>
<i>L. riparium</i> -L	61 $\pm$ 5.82 <sup>c</sup>	9 $\pm$ 1.72 <sup>c</sup>	105.6 $\pm$ 7.1 <sup>c</sup>	19.3 $\pm$ 3.33 <sup>c</sup>	71.8 $\pm$ 4.4 <sup>b</sup>	14.2 $\pm$ 3.33 <sup>c</sup>
<i>B. erythrorrhizon</i> -Z	61.3 $\pm$ 4.55 <sup>c</sup>	8.1 $\pm$ 1.84 <sup>d</sup>	98 $\pm$ 4.74 <sup>d</sup>	14.1 $\pm$ 2.49 <sup>a</sup>	72.3 $\pm$ 4.22 <sup>b</sup>	10.2 $\pm$ 2.24 <sup>b</sup>
<i>R. murale</i> -L	53.5 $\pm$ 9.15 <sup>d</sup>	10.6 $\pm$ 2.29 <sup>c</sup>	75 $\pm$ 4.49 <sup>c</sup>	16.2 $\pm$ 2.79 <sup>d</sup>	32.9 $\pm$ 6.8 <sup>c</sup>	5.4 $\pm$ 1.69 <sup>d</sup>
<i>E. praelongum</i> -Z	26.7 $\pm$ 4.3 <sup>c</sup>	3.9 $\pm$ 1 <sup>f</sup>	44.1 $\pm$ 4.74 <sup>f</sup>	8.1 $\pm$ 1.57 <sup>c</sup>	33.4 $\pm$ 2.85 <sup>c</sup>	5.1 $\pm$ 0.95 <sup>d</sup>
<i>E. praelongum</i> -L	19.9 $\pm$ 2.74 <sup>f</sup>	1.8 $\pm$ 0.36 <sup>g</sup>	34 $\pm$ 4.49 <sup>g</sup>	8.8 $\pm$ 3.45 <sup>c</sup>	25.6 $\pm$ 4.78 <sup>d</sup>	4.3 $\pm$ 1.15 <sup>c</sup>

(S.P.L: sporophyte Plant length, S.L: seta length, C.L: capsule length, L.L: leaf length, CO.L: costa length, CO.W: costa width, B.C.L: length of basal cells in the leaf, B.C.L/B.C.W: ratio of basal cells length to width, L.C.L: length of lamina cells in the leaf, L.C.L/L.C.W: ratio of lamina cells length to width, A.C.L: length of apical cells in the leaf, A.C.L/A.C.W: ratio of apical cells length to width)

**Table 6.** Statistical study of some morphological criteria for acrocarpous species (1).

Species	S.P.L/mm	S.L/mm	C.L/mm	L.L/mm	CO.L/mm	CO.W/ $\mu$ m
<i>S. ruralis</i> -L	19.7 $\pm$ 0.85 <sup>a</sup>	16.4 $\pm$ 0.77 <sup>a</sup>	3.31 $\pm$ 0.28 <sup>a</sup>	3.31 $\pm$ 0.17 <sup>a</sup>	3.31 $\pm$ 0.17 <sup>a</sup>	56 $\pm$ 4.82 <sup>a</sup>
<i>S. ruralis</i> -Z	22 $\pm$ 1.73 <sup>b</sup>	17.1 $\pm$ 1.52 <sup>b</sup>	4.94 $\pm$ 0.48 <sup>b</sup>	4.14 $\pm$ 0.22 <sup>b</sup>	4.14 $\pm$ 0.22 <sup>b</sup>	52 $\pm$ 4.94 <sup>a</sup>
<i>To. fragilis</i> -L	12.5 $\pm$ 0.65 <sup>c</sup>	10.5 $\pm$ 0.62 <sup>c</sup>	2.08 $\pm$ 0.16 <sup>c</sup>	2.27 $\pm$ 0.12 <sup>c</sup>	2.27 $\pm$ 0.11 <sup>c</sup>	75 $\pm$ 4.95 <sup>b</sup>
<i>Bar. unguiculata</i> -Z	21.4 $\pm$ 1.09 <sup>b</sup>	16.5 $\pm$ 0.99 <sup>a</sup>	4.9 $\pm$ 0.79 <sup>b</sup>	2.86 $\pm$ 0.19 <sup>d</sup>	2.86 $\pm$ 0.19 <sup>d</sup>	90 $\pm$ 7.56 <sup>c</sup>
<i>Ti. anomala</i> -L	23.5 $\pm$ 2.11 <sup>d</sup>	18.2 $\pm$ 2.04 <sup>d</sup>	5.3 $\pm$ 0.35 <sup>d</sup>	4.48 $\pm$ 0.21 <sup>e</sup>	4.48 $\pm$ 0.21 <sup>e</sup>	212 $\pm$ 9.15 <sup>d</sup>
<i>D. fallax</i> -Z	20.2 $\pm$ 0.97 <sup>a</sup>	17.6 $\pm$ 0.93 <sup>b</sup>	2.59 $\pm$ 0.26 <sup>e</sup>	2.42 $\pm$ 0.12 <sup>f</sup>	2.42 $\pm$ 0.12 <sup>f</sup>	102 $\pm$ 15.9 <sup>e</sup>
<i>Sch. apocarpium</i> -L	3.7 $\pm$ 0.15 <sup>e</sup>	1.4 $\pm$ 0.1 <sup>e</sup>	2.31 $\pm$ 0.09 <sup>f</sup>	2.59 $\pm$ 0.2 <sup>g</sup>	2.59 $\pm$ 0.2 <sup>g</sup>	77 $\pm$ 6.56 <sup>b</sup>
<i>Sch. apocarpium</i> -Z	2.6 $\pm$ 0.1 <sup>f</sup>	1.1 $\pm$ 0.09 <sup>e</sup>	1.52 $\pm$ 0.06 <sup>g</sup>	2.47 $\pm$ 0.16 <sup>f</sup>	2.47 $\pm$ 0.16 <sup>f</sup>	80 $\pm$ 7.62 <sup>b</sup>
<i>G. pulvinata</i> -L	5.3 $\pm$ 0.38 <sup>g</sup>	3.8 $\pm$ 0.33 <sup>f</sup>	1.54 $\pm$ 0.12 <sup>g</sup>	2.44 $\pm$ 0.21 <sup>f</sup>	2.44 $\pm$ 0.21 <sup>f</sup>	60 $\pm$ 5.56 <sup>a</sup>
<i>G. pulvinata</i> -Z	5 $\pm$ 0.32 <sup>g</sup>	3.1 $\pm$ 0.24 <sup>g</sup>	1.91 $\pm$ 0.14 <sup>h</sup>	2.26 $\pm$ 0.17 <sup>c</sup>	2.26 $\pm$ 0.17 <sup>c</sup>	59 $\pm$ 5.52 <sup>a</sup>
<i>Bry. caespitium</i> -L	16.1 $\pm$ 0.7 <sup>h</sup>	14 $\pm$ 0.676 <sup>h</sup>	2.06 $\pm$ 0.12 <sup>c</sup>	1.32 $\pm$ 0.05 <sup>h</sup>	1.32 $\pm$ 0.05 <sup>h</sup>	55 $\pm$ 4.99 <sup>a</sup>
<i>Bry. caespitium</i> -Z	19.5 $\pm$ 1.1 <sup>a</sup>	17.2 $\pm$ 1.1 <sup>b</sup>	2.3 $\pm$ 0.18 <sup>f</sup>	1.52 $\pm$ 0.13 <sup>i</sup>	1.52 $\pm$ 0.13 <sup>i</sup>	85 $\pm$ 7.3 <sup>f</sup>
<i>Bry. capillare</i> -L	24.9 $\pm$ 1.62 <sup>i</sup>	20.7 $\pm$ 1.54 <sup>i</sup>	4.15 $\pm$ 0.23 <sup>i</sup>	3.31 $\pm$ 0.19 <sup>a</sup>	3.31 $\pm$ 0.19 <sup>a</sup>	111 $\pm$ 13.52 <sup>g</sup>
<i>Bry. capillare</i> -Z	25 $\pm$ 3.32 <sup>i</sup>	20.7 $\pm$ 3.27 <sup>i</sup>	4.31 $\pm$ 0.26 <sup>j</sup>	2.89 $\pm$ 0.19 <sup>d</sup>	2.6 $\pm$ 0.17 <sup>g</sup>	127 $\pm$ 9 <sup>h</sup>
<i>Bry. argenteum</i> -L	16.1 $\pm$ 1.65 <sup>h</sup>	14.7 $\pm$ 1.64 <sup>j</sup>	1.44 $\pm$ 0.16 <sup>g</sup>	0.99 $\pm$ 0.03 <sup>j</sup>	0.6 $\pm$ 0.02 <sup>j</sup>	52 $\pm$ 3.91 <sup>a</sup>
<i>Bry. argenteum</i> -Z	16 $\pm$ 1.83 <sup>h</sup>	14 $\pm$ 1.82 <sup>h</sup>	1.98 $\pm$ 0.26 <sup>c,h</sup>	0.94 $\pm$ 0.07 <sup>j</sup>	0.56 $\pm$ 0.04 <sup>j</sup>	48 $\pm$ 5.25 <sup>i</sup>

(S.P.L: sporophyte Plant length, S.L: seta length, C.L: capsule length, L.L: leaf length, CO.L: costa length, CO.W: costa width)

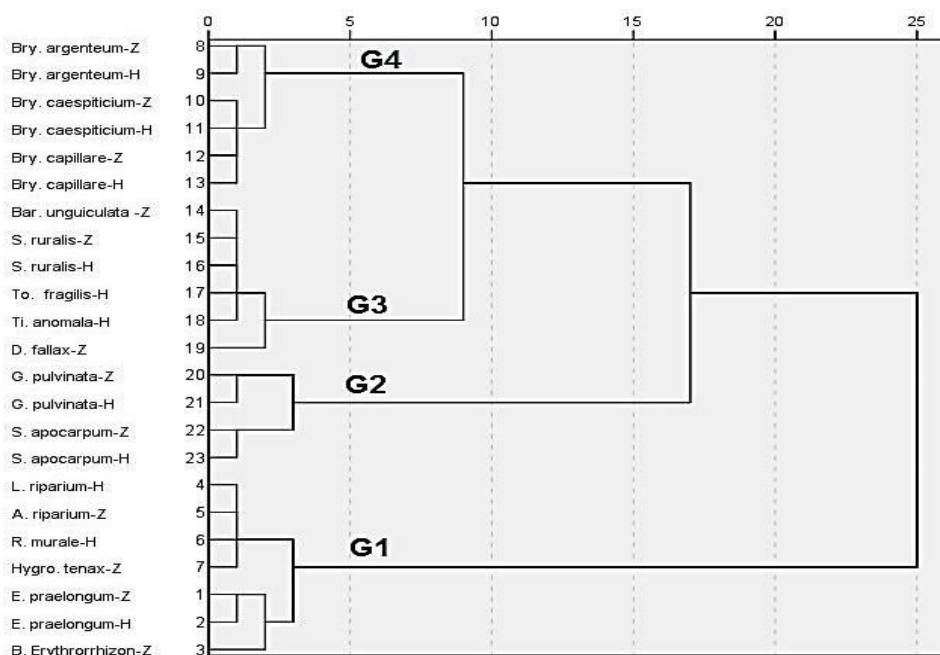
**Table 7.** Statistical study of some morphological criteria for acrocarpous species (2).

Species	B.C.L/ $\mu$ m	B.C.L/B.C.W	L.C.L/ $\mu$ m	L.C.L/L.C.W	A.C.L/ $\mu$ m	A.C.L/A.C.W
<i>S. ruralis</i> -L	52.7 $\pm$ 7.99 <sup>a</sup>	3.5 $\pm$ 0.46 <sup>a</sup>	11.9 $\pm$ 1.19 <sup>a</sup>	1.15 $\pm$ 0.17 <sup>a</sup>	9.7 $\pm$ 1.3 <sup>a</sup>	1.11 $\pm$ 0.21 <sup>a</sup>
<i>S. ruralis</i> -Z	48.5 $\pm$ 5 <sup>b</sup>	2.9 $\pm$ 0.54 <sup>b</sup>	11.7 $\pm$ 1.4 <sup>a</sup>	1.1 $\pm$ 0.16 <sup>a</sup>	12.4 $\pm$ 1.71 <sup>b</sup>	1.2 $\pm$ 0.17 <sup>a,b</sup>
<i>To. fragilis</i> -L	52.8 $\pm$ 6.38 <sup>a</sup>	6.2 $\pm$ 1.22 <sup>c</sup>	8.1 $\pm$ 1.08 <sup>b</sup>	1.09 $\pm$ 0.26 <sup>a</sup>	7.7 $\pm$ 0.79 <sup>c</sup>	1.1 $\pm$ 0.27 <sup>a</sup>
<i>Bar. unguiculata</i> -Z	24.3 $\pm$ 2.58 <sup>c</sup>	1.8 $\pm$ 0.22 <sup>d,f</sup>	14.4 $\pm$ 1.11 <sup>c,d</sup>	1.2 $\pm$ 0.19 <sup>a</sup>	12.3 $\pm$ 1.1 <sup>b</sup>	1.2 $\pm$ 0.14 <sup>a,b</sup>
<i>Ti. anomala</i> -L	39.2 $\pm$ 2.24 <sup>d</sup>	3.4 $\pm$ 0.41 <sup>a</sup>	6.5 $\pm$ 1.15 <sup>b</sup>	1.15 $\pm$ 0.28 <sup>a</sup>	7.4 $\pm$ 0.85 <sup>c</sup>	1.2 $\pm$ 0.27 <sup>a,b</sup>
<i>D. fallax</i> -Z	14.8 $\pm$ 3.62 <sup>e</sup>	1.6 $\pm$ 0.51 <sup>d</sup>	11.3 $\pm$ 1.62 <sup>a</sup>	1.45 $\pm$ 0.33 <sup>b</sup>	8.4 $\pm$ 1.73 <sup>c</sup>	1.3 $\pm$ 0.4 <sup>b</sup>
<i>Sch. apocarpium</i> -L	30.7 $\pm$ 3.71 <sup>f</sup>	2.7 $\pm$ 0.39 <sup>e</sup>	11.7 $\pm$ 1.69 <sup>a</sup>	1.3 $\pm$ 0.23 <sup>c</sup>	12.2 $\pm$ 1.62 <sup>b</sup>	1.3 $\pm$ 0.27 <sup>b</sup>
<i>Sch. apocarpium</i> -Z	21.6 $\pm$ 2.52 <sup>g</sup>	1.9 $\pm$ 0.27 <sup>f</sup>	7.8 $\pm$ 0.8 <sup>b</sup>	1.14 $\pm$ 0.22 <sup>a</sup>	11 $\pm$ 1.17 <sup>a,b</sup>	1.3 $\pm$ 0.2 <sup>b</sup>
<i>G. pulvinata</i> -L	33.7 $\pm$ 3.68 <sup>h,i</sup>	2.5 $\pm$ 0.33 <sup>e</sup>	12.1 $\pm$ 1.18 <sup>a</sup>	1.1 $\pm$ 0.17 <sup>a</sup>	10.1 $\pm$ 1 <sup>a</sup>	1.1 $\pm$ 0.19 <sup>a</sup>
<i>G. pulvinata</i> -Z	23.1 $\pm$ 2.89 <sup>c,g</sup>	2.1 $\pm$ 0.36 <sup>g</sup>	13.1 $\pm$ 1.29 <sup>d</sup>	1.2 $\pm$ 0.18 <sup>a</sup>	10 $\pm$ 0.99 <sup>a</sup>	1.1 $\pm$ 0.17 <sup>a</sup>
<i>Bry. caespitium</i> -L	40.4 $\pm$ 4.96 <sup>d</sup>	2.6 $\pm$ 0.51 <sup>e</sup>	61.8 $\pm$ 7.88 <sup>c</sup>	5.3 $\pm$ 0.85 <sup>d</sup>	46.3 $\pm$ 3.92 <sup>d</sup>	5.7 $\pm$ 0.71 <sup>c</sup>
<i>Bry. caespitium</i> -Z	34.5 $\pm$ 2.83 <sup>i</sup>	2.3 $\pm$ 0.29 <sup>g</sup>	58.8 $\pm$ 4.77 <sup>f</sup>	6 $\pm$ 1.1 <sup>e</sup>	42.7 $\pm$ 2.39 <sup>e</sup>	5 $\pm$ 0.73 <sup>d</sup>
<i>Bry. capillare</i> -L	75.4 $\pm$ 5.94 <sup>j</sup>	3.8 $\pm$ 0.43 <sup>h</sup>	57.1 $\pm$ 9.06 <sup>g</sup>	2.6 $\pm$ 0.47 <sup>f</sup>	49.2 $\pm$ 5.24 <sup>f</sup>	2.7 $\pm$ 0.36 <sup>e</sup>
<i>Bry. capillare</i> -Z	74.8 $\pm$ 5.42 <sup>j</sup>	4.1 $\pm$ 0.66 <sup>i</sup>	63.8 $\pm$ 4.11 <sup>h</sup>	3.3 $\pm$ 0.43 <sup>g</sup>	63.3 $\pm$ 5.4 <sup>g</sup>	3.2 $\pm$ 0.47 <sup>f</sup>
<i>Bry. argenteum</i> -L	31.5 $\pm$ 2.91 <sup>h,i</sup>	1.8 $\pm$ 0.25 <sup>d,f</sup>	55.1 $\pm$ 5.93 <sup>i</sup>	5.5 $\pm$ 0.97 <sup>h</sup>	66.3 $\pm$ 3.24 <sup>h</sup>	5.3 $\pm$ 0.71 <sup>g</sup>
<i>Bry. argenteum</i> -Z	43.8 $\pm$ 5.06 <sup>k</sup>	3.6 $\pm$ 0.64 <sup>a</sup>	52.1 $\pm$ 4.1 <sup>j</sup>	5 $\pm$ 0.83 <sup>i</sup>	64.9 $\pm$ 6.57 <sup>i</sup>	5.8 $\pm$ 1.05 <sup>e</sup>

(B.C.L: length of basal cells in the leaf, B.C.L/B.C.W: ratio of basal cells length to width, L.C.L: length of lamina cells in the leaf, L.C.L/L.C.W: ratio of lamina cells length to width, A.C.L: length of apical cells in the leaf, A.C.L/A.C.W: ratio of apical cells length to width)

Hierarchical cluster analysis of morphological characters grouped the studied species into four distinct clusters, which corresponded directly to their taxonomic orders (Figure 3). The classification was as follows: the first group contained pleurocarpous mosses of the order Hypnales (*Amblystegium riparium*, *Leptodictyum riparium*, *Hygroamblystegium tenax*, *Brachythecium erythrorrhizon*, *Eurhynchium praelongum*, and

*Rhynchostegium murale*). The second group comprised two species of Grimmiaceae (*Grimmia pulvinata* and *Schistidium apocarpum*). The third group included five species of Pottiaceae (*Barbula unguiculata*, *Timmiella anomala*, *Tortella fragilis*, *Didymodon fallax*, and *Syntrichia ruralis*). Finally, the fourth group comprised three Bryales species: *Bryum argenteum*, *B. capillare*, and *B. caespitium*.



**Figure 3.** Dendrogram of the species based on morphological criteria using SPSS V22 (Hierarchical Clustering). (The letter at the end of each species name denotes the collection area: 'H' denotes Al-Haffah region and 'Z' denotes Al-Zabadani region).

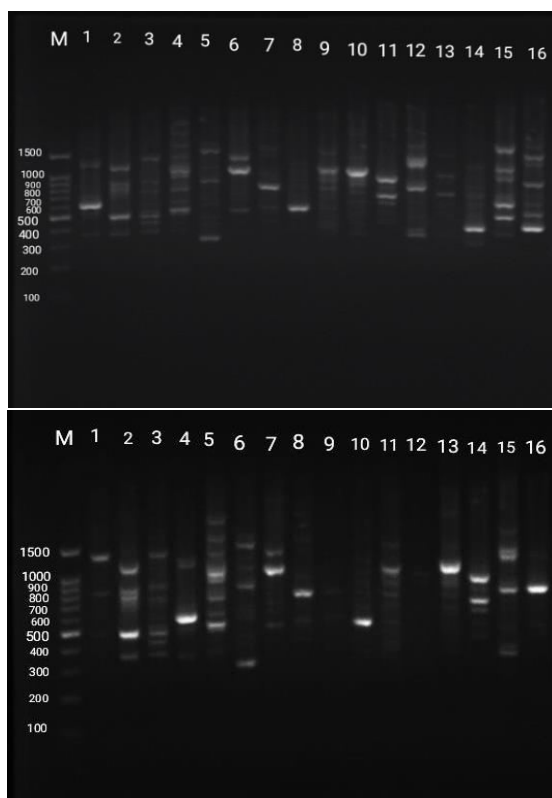
### 3.3. Molecular analysis

Molecular characterization was conducted to study the genetic distance between moss species found in Al-Zabadani and Al-Haffah regions. DNA concentration in pleurocarpous moss species was higher when compared to acrocarpous species, with values exceeding  $100 \text{ ng} \cdot \mu\text{l}^{-1}$  for all pleurocarpous species, while acrocarpous species had concentrations below  $100 \text{ ng} \cdot \mu\text{l}^{-1}$  (Table 8). This difference is attributed to the smaller amount of plant material from which DNA was isolated in acrocarpous species in comparison to pleurocarpous species. DNA samples had A260/280 ratios higher than 1.8, indicating high purity. Furthermore, the results confirmed that CTAB method was highly efficient for extracting DNA from moss samples, as supported by several previous studies (Schlink and Reski, 2002; Baraki *et al.*, 2018).

**Table 8.** DNA concentration and purity of the studied Moss Species.

Species	Purity	Concentration $\text{ng} \cdot \mu\text{l}^{-1}$	
Pleurocarpous moss species	<i>Rhynchostegium murale</i>	1.96	481.8
	<i>Eurhynchium praelongum</i>	1.82	341.8
	<i>Leptodictyum riparium</i>	2	130.1
	<i>Amblystegium riparium</i>	1.94	125.7
	<i>Brachythecium erythrorrhizon</i>	1.99	118.5
	<i>Hygroamblystegium tenax</i>	1.94	112
Acrocarpous moss species	<i>Grimmia pulvinata</i>	1.84	76.5
	<i>Bryum caespiticium</i>	1.92	52.4
	<i>Bryum capillare</i>	1.94	48
	<i>Didymodon fallax</i>	1.78	44.6
	<i>Schistidium apocarpium</i>	1.98	36.1
	<i>Tortella fragilis</i>	1.81	35.7
	<i>Syntrichia ruralis</i>	1.78	26.3
	<i>Barbula unguiculata</i>	2.1	25.4
	<i>Bryum argenteum</i>	1.97	22.8
<i>Timmia anomala</i>	1.84	19.5	

We employed the Random Amplified Polymorphic DNA (RAPD) technique for molecular characterization. It was accomplished using 10 primers produced by Alpha DNA, all of which yielded reliable results in elucidating the genetic variation among the studied species. Figure 4 shows the electrophoresis results on a 1.8% agarose gel using primers OP-Z13 and OP-R11.



**Figure 4.** Gel Electrophoresis results using primers OP-Z13 and OP-R11.

(Molecular weights on the left, at the top the studied species as follows: 1: *A. riparium*, 2: *E. praelongum*, 3: *Sch. Apocarpium*, 4: *G. pulvinata*, 5: *Bar. unguiculata*, 6: *D. fallax*, 7: *Ti. anomala*, 8: *To. fragilis*, 9: *Bry. argenteum*, 10: *Bry. capillare*, 11: *R. murale*, 12: *Bry. caespiticium*, 13: *Hygro. tenax*, 14: *S. ruralis*, 15: *L. riparium*, 16: *B. erythrorrhizon*)

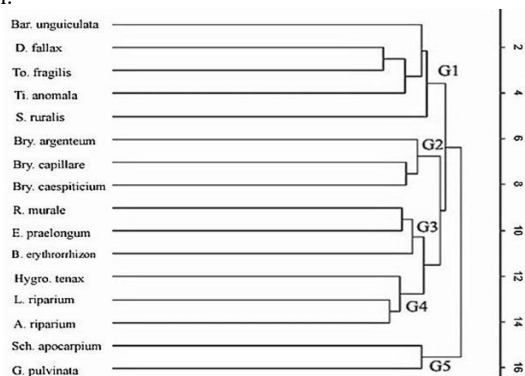
Table 9 presents amplification results using RAPD, showing a total of 163 molecular markers, with an average of 16.3 markers per primer. The total number of bands was 701, averaging 70.1 bands per primer. The table also indicates that primer OP-R11 yielded the highest number of molecular markers, totaling 25 markers and 120 bands. However, primers OP-E18 and OP-E12 yielded the lowest number of molecular markers, with 12 markers each. PIC values were consistent across all primers, ranging from 0.28 to 0.37, with an average of 0.34. These results indicate the efficiency of RAPD technique as well as the primers employed in this study to reveal the genetic diversity among species.

**Table 9.** Number of molecular markers and bands yielded by each primer, with PIC values.

Num	Primer Code	Number of molecular markers	Number of Resulting Bands	PIC
1	OP-Z14	17	67	0.35
2	OP-R15	15	74	0.35
3	OP-R11	25	120	0.36
4	OP-R07	18	56	0.28
5	OP-E12	12	53	0.37
6	OP-E01	14	47	0.31
7	OP-E18	12	56	0.37
8	OP-Z13	19	118	0.36
9	OP-R04	14	57	0.33
10	OP-R08	17	53	0.29
Total		163	701	3.37
Average		16.3	70.1	0.34

Hierarchical cluster analysis (Dendrogram) was conducted using Past Version 1.92 software to determine genetic similarity or divergence among the studied species, based on the presence or absence of bands resulting from RAPD amplification. The dendrogram analysis revealed the distribution of species into five groups (clusters) according to their respective families (Figure 5). The first group included five Pottiaceae species: *Barbula unguiculata*, *Tortella fragilis*, *Timmia anomala*, *Didymodon fallax*, and *Syntrichia ruralis*. The second group comprised three species of Bryaceae: *Bryum caespiticium*, *B. capillare*, and *B. argenteum*. The third group included three species of pleurocarpous mosses belonging to Brachytheciaceae: *Eurhynchium praelongum*, *Rhynchostegium murale*, and *Brachythecium erythrorrhizon*. The fourth group also contained three species of pleurocarpous mosses, but belonging to Amblystegiaceae: *Leptodictyum riparium*, *Hygroamblystegium tenax*, and *Amblystegium riparium*. Finally, the fifth group consisted of two Grimmiaceae species: *Schistidium apocarpum* and *Grimmia pulvinata*.

Cluster analysis results of molecular and morphological data yielded congruent results, both grouping the studied species according to their established orders and families. While morphological clustering effectively delineated taxa at the ordinal level, molecular data provided finer resolution, clearly distinguishing species at the family level.



**Figure 5.** Dendrogram representing the studied species based on the Jaccard coefficient (paired groups algorithm).

## 4. Discussion

Syria's diverse landscapes provide rich habitats for a wide array of plant species, including bryophytes. However, the taxonomic study of Syrian mosses remains notably limited. This gap highlights the significant potential of regional surveys, especially in humid areas with conditions favorable for moss growth, to yield new species records and substantially enrich our understanding of the national flora.

### 4.1. Climatic Impact on Moss Distribution

Our analysis of climate data reveals a clear contrast between the two study regions. Al-Haffah experiences high humidity and extended rainfall for most of the year, with only a short three-month dry interval (Saeed and Hasso, 2021). In contrast, Al-Zabadani endures a more extended dry period of five months (Ismaeel, 2017). The significant differences in climate are directly mirrored by variations in moss diversity and abundance. The consistently humid conditions in Al-Haffah foster a significantly richer and more abundant moss community than the drier environment of Al-Zabadani; these findings align with Tizini *et al.* (2012) and Zhang *et al.* (2023), who identified moisture availability as a primary factor governing moss establishment and proliferation. Furthermore, as Al-Araj (2007) suggested, the favorable conditions "including optimal temperature, high humidity, and ample precipitation" enhance critical developmental stages like protonemal rhizoid formation, thereby supporting new gametophyte growth. The denser vascular plant canopy in Al-Haffah, also noted for its role in moisture retention and protection from solar radiation, creates a microclimate that further amplifies this effect (Al-Araj, 2007; Zhang *et al.*, 2023).

### 4.2. Morphological Diversity and Phenotypic Clustering

Statistical evaluation of the morphological data revealed significant phenotypic variation, not only among different species but also within populations of the same species across the two regions. This points to a strong environmental influence on morphological criteria, driven by factors like topography, rainfall, and humidity. Hierarchical clustering based on these morphological criteria grouped the species into four distinct clusters, which aligned well with established taxonomic orders. Hierarchical clustering based on these morphological traits grouped the species into four distinct clusters, which aligned well with established taxonomic orders. This congruence with standard morphological classifications validates the utility of our selected morphological criteria for reliable species grouping and differentiation (Goffinet and Shaw, 2008).

### 4.3. Genetic Variation and Molecular Marker Efficacy

Molecular characterization of moss species from Al-Zabadani and Al-Haffah regions revealed significant insights into their genetic variation. Our data consistently show that species adapted to dry environments contain minimal DNA, unlike their moisture-dependent relatives. This pattern indicates that a reduced genome has been selectively advantageous in arid habitats. Furthermore, the higher DNA yield we obtained from pleurocarpous species is likely a methodological artifact. Their distinctive growth

forms simply provide more biomass for extraction and should not be misinterpreted as evidence of a larger genome size (Li *et al.*, 2023). The high purity of DNA samples supports the effectiveness of CTAB method, which is consistent with previous studies that highlight its superiority over commercial kits in terms of both concentration and purity (Schlink and Reski, 2002). Our results confirm that RAPD is an effective method for detecting genetic variation in mosses. The primers produced a rich set of molecular markers, consistent with previous applications of this technique. For instance, Soni *et al.* (2014) successfully used RAPD to reveal genetic patterns in *Plagiochasma appendiculatum* across Himalayan elevation gradients. Similarly, Dale *et al.* (1999) demonstrated its effectiveness in detecting variation among *Hennediella heimii* populations from ecologically distinct regions. The molecular data grouped the species into five distinct genetic clusters, which largely matched their existing family level classifications. This agreement between DNA data and morphological characteristics also seen in studies on *Didymodon manhanensis* and *Syntrichia* spp. highlights how combining molecular methods with traditional taxonomy gives us a more complete picture (Gallego *et al.*, 2022; Feng *et al.*, 2022). Together, these approaches create a stronger foundation for defining species boundaries and understanding evolutionary relationships, leading to a more accurate classification system.

### 4.4. Broader Implications and Future Perspectives

Our results provide valuable insights into the geographic distribution patterns of mosses in Syria. The distinct genetic clusters found between the two regions support the idea that geographic isolation is a key driver in shaping moss biodiversity, which helps us better understand the evolutionary history of plants in the Mediterranean basin. While our findings are promising, they should be considered in light of some limitations. The relatively small number of sampling sites and limited sample sizes from certain populations may affect how broadly our results can be generalized. Furthermore, while the RAPD technique was effective for revealing genetic variation, newer high-resolution sequencing technologies (e.g., RAD-seq, SSR, ISSR) could provide a more detailed understanding of the genomic architecture underlying adaptation. Building on these results, we propose several promising future directions. First, a comprehensive national survey is urgently needed to fully document and map Syria's bryophyte diversity, especially in unexplored regions. Second, integrating our data with phylogeographic models would help reconstruct predict their responses to future environmental change.

## 5. Conclusion

This study addresses a critical gap in the floristic knowledge of Syria by providing the first comprehensive assessment of moss diversity across two distinct biogeographical regions, Al-Zabadani (Damascus countryside) and Al-Haffah (Latakia). Sixteen moss species were identified, and analysis revealed a clear correlation between their distribution and local environmental gradients, offering a baseline model for predicting bryophyte responses to ecological changes. The

high concordance between morphological and molecular cluster analyses validates the use of classical taxonomic methods while revealing the superior precision of molecular markers in distinguishing species at the family level. This dual-methodological approach provides taxonomists with a validated framework for accurate species identification, especially for morphologically variable taxa. For ecologists, the clear relationship between species distribution and environmental conditions provides a powerful tool for biomonitoring and assessing habitat quality. The successful application of the RAPD technique and the specific primers confirmed their high efficiency in detecting intraspecific genetic variation. The successful application of the RAPD technique and the specific primers confirmed their high efficiency in detecting intraspecific genetic variation. To build on these findings, future studies should broaden sampling to cover additional Syrian ecoregions and integrate additional molecular markers, such as ITS or rbcL. This will enable the construction of a comprehensive DNA barcode library for Syrian bryophytes, ultimately leading to a more robust phylogenetic understanding and effective conservation planning for this critical component of plant diversity.

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