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Diversity and phylogenetic relationships among *Moniezia* spp. (Cestoda: Anoplocephalidae): An Inference from *COX1* and *SSU rDNA* Sequences

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Abstract: Introduction: *Moniezia* species are common tapeworms infecting domestic ruminants worldwide. However, their morphological similarities make species-level identification challenging, often leading to diagnostic confusion among veterinarians. Studies on the population dynamics of these parasites remain scarce in many regions, including Senegal, a West African country. **Methods:** We investigated the diversity, population structure, and dynamics of the genus *Moniezia* using 29 mitochondrial cytochrome c oxidase subunit 1 *(Cox1*) sequences and 22 nuclear small subunit ribosomal *RNA (SSU rDNA*) sequences. These were obtained from three species: *Moniezia expansa*, *Moniezia benedeni*, and *Moniezia* sp., collected from domestic ruminants (sheep, goats, and cattle) at Dakar's main slaughterhouse, SOGAS (formerly SERAS), between June 2013 and May 2014. Sequence alignment was performed using BioEdit, and genetic analyses were conducted with DnaSP, MEGA, Arlequin, and Network software. **Results:** Polymorphism analysis revealed that *M. benedeni* exhibited the highest genetic diversity (378 polymorphic sites in *SSU rDNA*), followed by *Moniezia* sp. (177 polymorphic sites in *Cox1*) and *M. expansa* (105 polymorphic sites in *Cox1*). The predominance of synonymous over non-synonymous mutations suggests the presence of purifying (negative) selection. Genetic structure analysis indicated clear differentiation between *M. expansa* and *M. benedeni*. Phylogenetic reconstruction showed that genetic variation was independent of host species and geographic origin. Furthermore, haplotype network analysis revealed evidence of cryptic species within the *Moniezia* genus. **Conclusion:** The *Moniezia* genus may represent a species complex, including *M. expansa, M. benedeni*, and potentially other yet undescribed species.

Keywords: population; *Moniezia*; population dynamic; evolution; structure; phylogeny

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1. Introduction

Domestic ruminant farming—including cattle, sheep, and goats—is practiced across all continents in a wide range of systems. In Senegal, it remains a deeply rooted traditional activity, engaging nearly one-third of households and almost half of the rural population [1]. Livestock farming plays a crucial role in food security, nutrition, wool and hide production, and rural employment. It is also a cornerstone of social relations and cultural identity in the country [1]. Accordingly, livestock development is one of the priority sectors of the Plan Senegal Emergent.

However, livestock production systems face numerous environmental challenges, including natural resource degradation, inadequate infrastructure, and the spread of parasitic diseases such as helminthiasis, which cause significant harm. In cases of severe infestation, helminths can lead to emaciation, stunted growth, reduced meat and milk yields, and deterioration in wool quality—resulting in substantial economic losses. Among these parasitic diseases, monieziosis is considered one of the most lethal [2–6]. Several species of Moniezia have been described [7–9], but only two are currently recognized as valid in domestic ruminants: *Moniezia expansa* (Rudolphi, 1810) and *Moniezia benedeni* (Moniez, 1879) [10]. These species are distinguished by the morphology of their interproglottidal glands—rosette-shaped in *M. expansa* and linear in *M. benedeni*—and by the shape of their eggs, which are triangular in *M. expansa* and tetragonal in *M. benedeni*. Other *Moniezia* species have been reported to lack interproglottidal glands entirely, and their eggs may be deformed, complicating morphological identification.

This morphological ambiguity presents a real challenge for accurate species identification [11]. Isoenzymatic analysis by [12] suggested that *M. expansa* and *M. benedeni* may represent species complexes, casting doubt on the reliability of interproglottidal glands as a genus-specific diagnostic feature.

Although some studies have explored the genetic diversity of *Moniezia* in domestic ruminants globally, most have focused on distinguishing *M. expansa* from *M. benedeni* [11,13–18]. A recent study identified a new species, *Moniezia denticulata*, in small domestic ruminants in India [9]. This species lacks interproglottidal glands and is considered a closed morphological variant of *Moniezia*.

Despite the social, economic, and veterinary significance of these parasites, major uncertainties remain regarding their taxonomy, population dynamics, and phylogenetic and phylogeographic evolution.

The objective of this study is to assess the diversity, genetic structure, and population dynamics of Moniezia species using mitochondrial *Cox1* and nuclear *SSU rDNA* gene markers.

2. Methods

2.1. Sampling and Identification of Cestodes

The *Moniezia* specimens used in this study were collected between June 2013 and May 2014 from domestic ruminants at the main slaughterhouse in Dakar (SOGAS). Tapeworms were carefully

extracted from the intestines and identified based on morphological characteristics following staining procedures, using established taxonomic keys [7,19–21]. Mature proglottids were stained with iron hydrochloric carmine. The proglottids were first fixed and rinsed in 70% ethanol, then stained, destained in acidified ethanol (100 mL of 70% ethanol with 2 mL concentrated HCl), dehydrated through a graded ethanol series, cleared with eugenol (clove oil), and mounted in Canada balsam. Stained specimens were examined and photographed using a Leitz photo research microscope.

For molecular analysis, fragments of each worm's proglottids were preserved in 95% ethanol and stored at −20 °C. In total, 274 tapeworm specimens were collected and processed for further genetic study.

2.2. Molecular Analyses

2.2.1. DNA Extraction, PCR and Sequencing

A total of 64 adult tapeworms were collected from hosts and portions of proglottids preserved in 95% ethanol until used for molecular analysis. DNA extraction was performed using the Qiagen Mini Kit for Blood and Tissue, following the manufacturer's instructions (Qiagen Company, Hilden, Germany; QIAmp® DNA Mini Kit (250); Cat. No. 51306).

2.2.2. Amplification of *Cox1* and SSU *rDNA*

The PCRs were performed in Senegal (Laboratory of Evolutionary Biology, Ecology and Ecosystem, Department of Animal Biology, Faculty of Science and Technology, Cheikh Anta DIOP University of Dakar, CP 10700, BP 5005 Dakar, Senegal) and sequencing reactions were performed in CNRGH at the National Center of Human Genomics Research (CNRGH, Evry, France). The *Cox1* gene was amplified using the following forward and reverse primers: Mo*Cox1*F (5'-CTGAGTGTTTTCAAAACATTTAG-3') and Mo*Cox1*R (5'-AAGCATGATGCA AAAGGCA-3'). PCRs were performed with a total volume of 22 µL containing 2.5 µL of DNA template, 1.5 µL of Mo*Cox1*F (10 µM), 1.5 µL of Mo*Cox1*R (10 µM), 2 µL of dNTPs (2.5 mM each), 1.6 µL of Buffer 10X, 0.15 µL of ExTaq HS and 12.75 µL of H_2O (Diop et al., 2015). The amplification conditions are summarized as initial heating to 94 °C for 3 min, followed by 35 cycles of amplification consisting of a denaturation step at 94 °C for 30 s, a hybridization step at 55 °C for 30 s and an elongation step at 72 °C for 50 s. A final elongation at 72 °C for 2 min was performed. For the *SSU rDNA* gene, the forward and reverse primers used were *SSU rDNA* PF (5'- CTATGG TTTATTGGATCATCTC-3') and *SSU rDNA* PR (5'-TCTAAATGATCAAGTTTGGTCGT-3'), with a 22 µL mix containing 5.0 µL of DNA template, 1.5 µL of each primer (10 µM), 2.0 µL of dNTPs (2.5 mM each), 1.6 µL of Buffer, 0.3 µL of ExTaq HS, and 10.1 µL of H_2O. The amplification conditions consist of initial heating to 98 °C for 10 s, followed by 35 cycles of amplification consisting of a denaturation step at 98 °C for 10 s, a hybridization step at 62 °C for 15 s and an elongation step at 68 °C for 2 min. A final elongation at 68 °C for 2 min was also performed. The PCR products were visualized on a 1.5% agarose gel. The amplicons were purified using BioGel P100 gels (Bio-Rad, Marnes-la-Coquette, France). Sequencing reactions (2 µL of PCR product) were performed using the Dye terminator v3.1 method in an ABI PRISMs 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequencing conditions were as follows: 96 °C for 5 min, 25 cycles of 96 °C for 10 s; 60 °C for 4 min and 15 °C indefinitely; products were purified with Sephadex G50 superfine columns (GE Healthcare). Alignment of acquired sequences and SNP discovery were performed using reference. Analysis was performed with Genalys version 2.0b software [22].

2.3. Phylogenetic and Phylogeographic Analyses

To investigate the genetic diversity and population dynamics of the genus *Moniezia*, Cox1 and *SSU rDNA* sequences previously obtained by our team [11] were combined with additional *Moniezia* sequences from various geographic regions available in the NCBI database. All sequences were

renamed and grouped according to species and genetic loci using BioEdit version 7.2.5 [23], which also facilitated sequence cleaning and alignment. Reference sequences used for alignment and comparison included AB821373 for the Cox1 gene and AB862301 for the *SSU rDNA* gene.

Genetic polymorphism, population structure, and demographic history were analyzed using MEGA version 11 [24], DnaSP version 5.10 [25], and Arlequin version 3.1.0.2 [26]. Haplotype networks were constructed using Network version 10.2.0.0 [27]. Demographic dynamics were assessed using Fu's Fs statistic and mismatch distribution analysis.

Phylogenetic reconstruction was performed using the maximum likelihood method. The most appropriate evolutionary model for the dataset was selected based on the corrected Akaike Information Criterion (AIC), implemented in MEGA version 11.

2.4. Moniezia Species Studied and Origin of Cox1 and SSU rDNA Sequences

Population genetic analyses of *Moniezia* spp. were based on nucleotide sequences of the mitochondrial *Cox1* and nuclear *SSU rDNA* genes obtained from *M. expansa*, *M. benedeni*, and *Moniezia* sp. Specimens were collected from domestic ruminants—sheep, goats, and cattle. In addition to our own data, *Cox1* and *SSU rDNA* sequences from other geographical regions, publicly available in the GenBank NCBI database, were incorporated into the analysis (Table 1).

In total, 29 *Cox1* and 22 *SSU rDNA* sequences representing *Moniezia* species from diverse localities were analyzed (Tables 1 and 2). Following sequence alignment, all positions containing gaps or missing data were excluded. The final dataset comprised 692 nucleotide sites for *Cox1* and 616 sites for *SSU rDNA*.

Table 1: *Cox1 sequences of Moniezia* spp.: origins, references and accession numbers.

Species of *Moniezia*	Number of Individuals Treated	Country	References	Access Numbers
Moniezia expansa	2	Ethiopia	[11]	AB821373 AB821374
	3	Senegal	[11]	AB821391 AB821392 AB821393
	1	China	[17]	MG099722
	1	Japan	[28]	AB099693
	1	Vietnam	[29]	LC422632
	1	Iraq	[30]	MH259793
Moniezia benedeni	4	Senegal	[11]	AB821394 AB821395 AB821396 AB821397
	2	Iraq	[30]	MH259796 MH259797
	2	Vietnam	[29]	LC422634 LC422636
	1	Japan	[28]	AB099692
Moniezia sp.	5	Failande	[31]	AY568213
			[17]	MG099713 MG099715 MG099716 MG099717
	1	South Africa	[17]	MG099713, MG099715, MG099716, MG099718
				MG099713 MG099715 MG099716 MG099719
	2	USA	[17]	MG099714 MG099718
	3	India	[32]	KJ576906
			[9]	OQ134465.1 OQ134466.1

Table 2: *SSU rDNA* sequences of *Moniezia* spp.: origins, authors and accession number.

Species of *Moniezia*	Number of Individuals Treated	Country	References	Access Numbers
Moniezia expansa	1	Ethiopia	[11]	AB862303
	3	Senegal	[11]	AB862300, AB862301, AB862302
	1	China	[33]	GU817405
Moniezia benedeni	1	Senegal	[11]	AB862304
	8	Iraq	[30]	MH173843, MH173844, MH173845, MH173846, MH173847, MH173848, MH203083, MH203084
	4	China	[33]	GU817401, GU817402, GU817403, GU817404
Moniezia sp.	4	India	[9]	OM296990, OM296991, OM296990.1, OM296991.1

3. Results

3.1. Polymorphism and Genetic Variability

The 29 *Cox1* sequences analyzed were grouped into 27 distinct haplotypes: 9 corresponding to *M. expansa*, 7 to *M. benedeni*, and 11 to *Moniezia* sp. Only two haplotypes (h10 and h13) included more than one *M. benedeni* sequence; all remaining haplotypes were private, each represented by a single individual (Table 3).

Table 3: Distribution of *Cox1* gene haplotypes according to localities.

Species of *Moniezia*	Haplotypes	Eth	SN	Ch	J	VN	TK	IN	IR	FL	SA	USA	Total
M. expansa	1	0	1	0	0	0	0	0	0	0	0	0	1
	2	0	1	0	0	0	0	0	0	0	0	0	1
	3	0	1	0	0	0	0	0	0	0	0	0	1
	4	1	0	0	0	0	0	0	0	0	0	0	1
	5	1	0	0	0	0	0	0	0	0	0	0	1
	6	0	0	1	0	0	0	0	0	0	0	0	1
	7	0	0	0	1	0	0	0	0	0	0	0	1
	8	0	0	0	0	1	0	0	0	0	0	0	1
	9	0	0	0	0	0	0	0	1	0	0	0	1
M. benedeni	10	0	2	0	0	0	0	0	0	0	0	0	2
	11	0	1	0	0	0	0	0	0	0	0	0	1
	12	0	1	0	0	0	0	0	0	0	0	0	1
	13	0	0	0	0	0	0	0	2	0	0	0	2
	14	0	0	0	0	1	0	0	0	0	0	0	1
	15	0	0	0	0	1	0	0	0	0	0	0	1
	16	0	0	0	1	0	0	0	0	0	0	0	1

Table 3: *Cont.*

Species of *Moniezia*	Haplotypes	Eth	SN	Ch	J	VN	TK	IN	IR	FL	SA	USA	Total
Moniezia sp.	17	0	0	0	0	0	0	0	0	1	0	0	1
	18	0	0	0	0	0	0	0	0	1	0	0	1
	19	0	0	0	0	0	0	0	0	1	0	0	1
	20	0	0	0	0	0	0	0	0	1	0	0	1
	21	0	0	0	0	0	0	0	0	1	0	0	1
	22	0	0	0	0	0	0	0	0	0	1	0	1
	23	0	0	0	0	0	0	0	0	0	0	1	1
	24	0	0	0	0	0	0	0	0	0	0	1	1
	25	0	0	0	0	0	0	1	0	0	0	0	1
	26	0	0	0	0	0	0	1	0	0	0	0	1
	27	0	0	0	0	0	0	1	0	0	0	0	1

Ch: China, Eth: Ethiopia, FL: Finland, IN: India, IR: Iraq, J: Japan, SA: South Africa, SN: Senegal, TK: Türkiye, USA: United States of America, VN: Vietnam.

The 22 *SSU rDNA* sequences were classified into 15 haplotypes: 2 for *M. expansa*, 9 for *M. benedeni*, and 4 for *Moniezia* sp. The most frequent haplotype comprised four individuals—three *M. expansa* from Senegal and one from China. Additionally, one haplotype grouped three *M. benedeni* individuals from China, while two other haplotypes included two *M. benedeni* individuals from Iraq. All other haplotypes were private and observed in single individuals (Table 4).

Table 4: Distribution frequencies of *SSU rDNA* gene haplotypes according to localities.

Species of *Moniezia*	Haplotypes	Eth	SN	Ch	IN	IR	FL	PL	Total
M. benedeni	1	0	0	3	0	0	0	0	3
	2	0	0	1	0	0	0	0	1
M. expansa	3	0	3	1	0	0	0	0	4
	4	1	0	0	0	0	0	0	1
Moniezia sp.	5	0	0	0	1	0	0	0	1
	6	0	0	0	1	0	0	0	1
M. benedeni	7	0	0	0	0	1	0	0	1
	8	0	0	0	0	2	0	0	2
	9	0	0	0	0	1	0	0	1
	10	0	0	0	0	1	0	0	1
	11	0	0	0	0	1	0	0	1
	12	0	0	0	0	2	0	0	2
	13	0	1	0	0	0	0	0	1
Moniezia sp.	14	0	0	0	1	0	0	0	1
	15	0	0	0	1	0	0	0	1

Ch: China, Eth: Ethiopia, FL: Finland, IN: India, IR: Iraq, P: Poland, SN: Senegal.

For the mitochondrial *Cox1* gene, a total of 206 polymorphic sites were identified across the dataset. The highest levels of polymorphism were observed in *Moniezia* sp. (177 sites) and *M. expansa* (105 sites). In all populations and subpopulations, the rate of non-synonymous substitutions (Kns) was lower than that of synonymous substitutions (Ks), indicating purifying selection. An exception was noted in *M. benedeni*, where Ks (0.006) and Kns (0.005) were nearly equivalent for the *Cox1* gene. All three subpopulations exhibited high haplotype diversity (Hd) and low nucleotide diversity (π), suggesting recent population expansion or selective sweeps (Table 5).

Table 5: Genetic variability of the *Cox1* gene.

Populations of *Moniezia*	Number of Individuals	S	h	Hd	Π	Ks	Kns
M. expansa	9	105	9	1.000	0.048	0.030	0.013
M. benedeni	9	64	7	0.944	0.043	0.005	0.006
Moniezia sp.	11	177	11	1.000	0.094	0.410	0.113
Overall population	29	206	27	0.995	0.090	0.088	0.019

h: number of haplotypes, Hd: haplotypic diversity, π: nucleotide diversity, Ks: synonym substitution type, Kns: non-synonymous substitution type, S: total number of polymorphic sites.

For the nuclear *SSU rDNA* gene, 406 polymorphic sites were detected in the overall population. Within subpopulations, *Moniezia* sp. exhibited 77 polymorphic sites, *M. benedeni* 378 sites, and *M. expansa* only 1 site. Across all groups, the Kns values were consistently lower than Ks, reinforcing the presence of purifying selection. Both haplotype diversity (Hd) and nucleotide diversity (π) were high in *M. expansa* and the other subpopulations, indicating substantial genetic variation (Table 6).

Table 6: Genetic variability of the *SSU rDNA* gene.

Populations of *Moniezia*	Number of Individuals	S	h	Hd	π	Ks	Kns
M. expansa	5	1	2	0.400	0.006	0.228	0.171
M. benedeni	13	378	9	0.936	0.280	0.218	0.138
Moniezia sp.	4	77	4	1.000	0.070	0.238	0.186
Overall population	22	406	15	0.952	0.235	0.226	0.164

h: number of haplotypes, Hd: haplotypic diversity, π: nucleotide diversity, Ks: synonym substitution type, Kns: non-synonymous substitution type, S: total number of polymorphic sites.

3.2. Genetic Structure

3.2.1. Genetic Differentiation

Nei's genetic distance found between *M. expansa* and *M. benedeni* (12.7%) was very close to that observed between *M. benedeni* and *Moniezia* sp. (12.8%) for the *Cox1* gene. These two distances were slightly greater than the distance between *M. expansa* and *Moniezia* sp. (10.7%). The genetic distance in intra-population was high in *Moniezia* sp. (11.2%) and low in *M. benedeni* (4.8%) and *M. expansa* (5.4%) (Table 7). For *SSU rDNA*, Nei's genetic distance between *M. benedeni* and *Moniezia* sp. was 45.6%. It was very close to that between *M. expansa* and *M. benedeni* (42.9%) and much greater than the distance between *M. expansa* and *Moniezia* sp. (5.2%). The intra-population genetic distance was greatest for *M. benedeni* (42.8%), followed by *Moniezia* sp. (8%) (Table 8).

Table 7: Nei intra- and inter-population genetic distances between species of the genus *Moniezia* for the *Cox1* gene.

Populations of *Moniezia*	Genetic distances			
	Intra Population	Inter Population		
		1	2	3
1. *M. expansa*	0.054	-	-	-
2. *M. benedeni*	0.048	0.127	-	-
3. *Moniezia* sp.	0.112	0.107	0.128	-

Table 8: Nei intra- and inter-population genetic distances between species of the genus *Moniezia* for the *SSU rDNA*.

Populations of *Moniezia*	Genetic Distances			
	Intra Population	Inter Population		
		1	2	3
1. *M. benedeni*	0.428	-	-	-
2. *M. expansa*	0.000	0.429	-	-
3. *Moniezia* sp.	0.080	0.456	0.052	-

3.2.2. Degree of Genetic Differentiation (Fst)

The overall *Fst* was highly significant for the *cox1* gene. The degree of differentiation between subpopulations was greatest between *M. expansa* and *M. benedeni* (0.573) (Table 9). For the *SSU rDNA* gene, the overall *Fst* was also highly significant. The *Fst* value between the *M. expansa* and *M. benedeni* subpopulations (0.473) was higher and far exceeded the *Fst* value between *M. expansa* and *Moniezia* sp. (0.113). The *Fst* value between *M. benedeni* and *Moniezia* sp. was 0.367 (Table 10).

Table 9: Degree of genetic differentiation (Fst) for the *Cox1* gene.

Populations of *Moniezia*	FST Between Populations	Global FST	*p-Value*
M. expansa/M. benedeni	0.573	**0.314**	**0.000**
M. expansa/Moniezia sp.	0.220		
M. benedeni/Moniezia sp.	0.358		

Table 10: Degree of genetic differentiation (Fst) for the *SSU rDNA* gene.

Populations of *Moniezia*	FST Between Populations	Global FST	*p*-Value
M. expansa/M. benedeni	0.473	0.150	0.000
M. expansa/Moniezia sp.	0.113		
M. benedeni/Moniezia sp.	0.367		

3.3. Demographic Evolution

For the *Cox1* gene, Fu's *Fs* was negative and insignificant for *M. expansa* and *Moniezia* sp., while it was positive and insignificant for *M. benedeni* (Table 11).

Table 11: Demo-genetic test for the *Cox1* gene.

Populations of *Moniezia*	Neutrality Index	
	Fu's *Fs*	*p*-Value
M. expansa	−0.552	0.233
M. benedeni	2.642	0.868
Moniezia sp.	−0.208	0.287
Overall population	0.627	0.462

The mismatch distribution curve plotted under the assumption of a population in equilibrium. Figures 1 and 2 show that the distribution is multimodal for both genes.

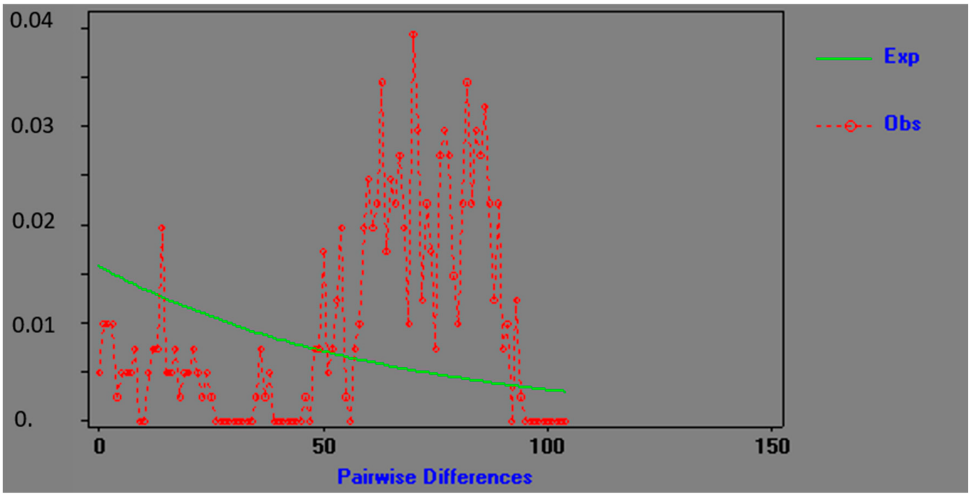

Figure 1: Observed distribution curve of pairwise nucleotide differences of *Cox1* gene sequence in the overall *Moniezia* population. Exp: Expected Obs: Observed.

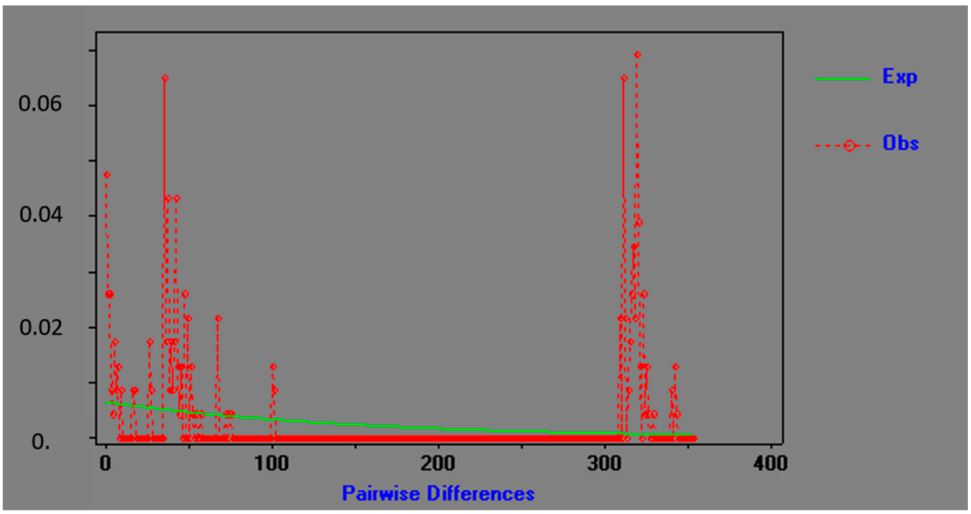

Figure 2: Observed distribution curve of pairwise nucleotide differences of *SSU rDNA* gene sequence in the overall *Moniezia* population. Exp: Expected Obs: Observed

3.4. Phylogenetic Approach

3.4.1. Phylogenetic Trees

Phylogenetic reconstruction based on the maximum likelihood method applied to the cox1 gene revealed that all *M. expansa* specimens clustered within a single clade. In contrast, *M. benedeni* formed two distinct clades: one comprising isolates from domestic ruminants (Bos taurus and Ovis aries), and another associated with Bubalus bubalis, representing wild ruminants. Similarly, Moniezia sp. segregated into two clades, both originating from wild ruminant hosts (Figure 3). The phylogenetic analysis of the *SSU rDNA* gene, conducted using the same methodology, yielded a comparable clade structure with consistently high bootstrap support values, except for a few poorly resolved branches (Figure 4).

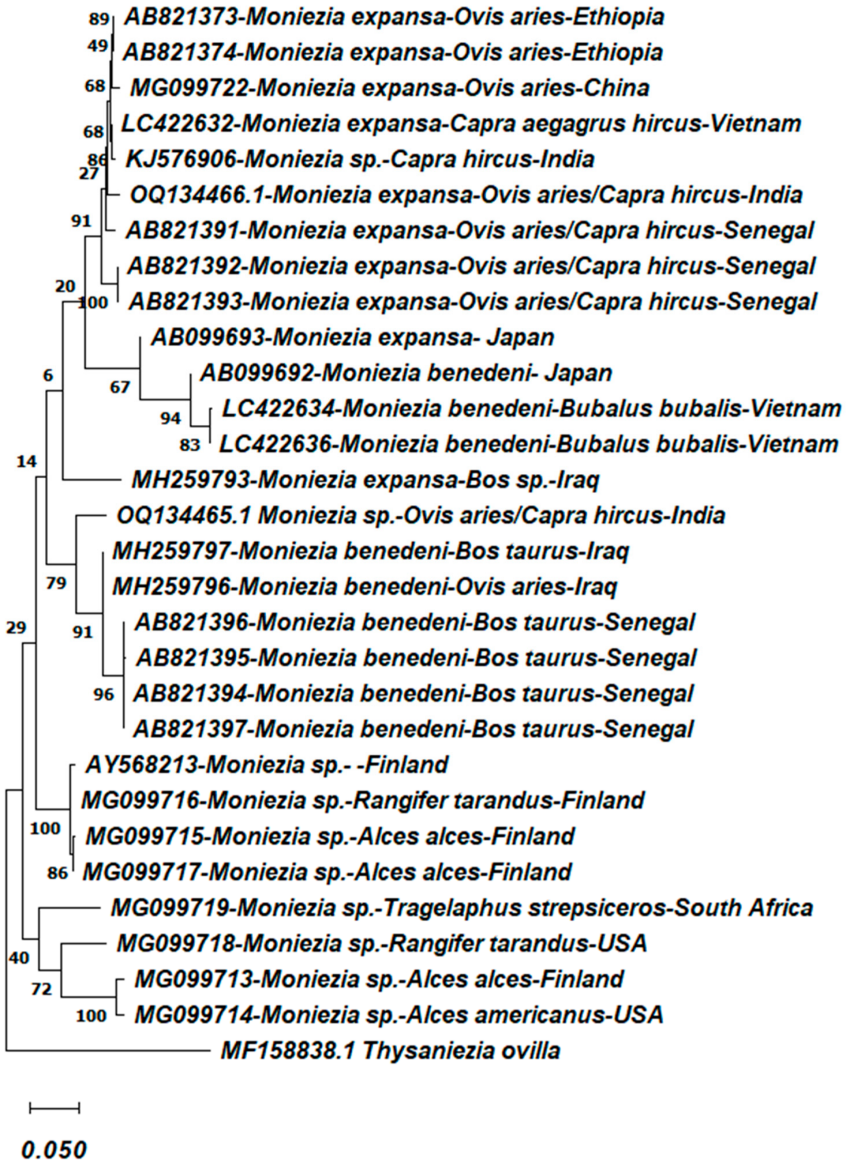

Figure 3: Phylogenetic tree obtained using the maximum likelihood method for the *Cox1* gene of *Moniezia*.

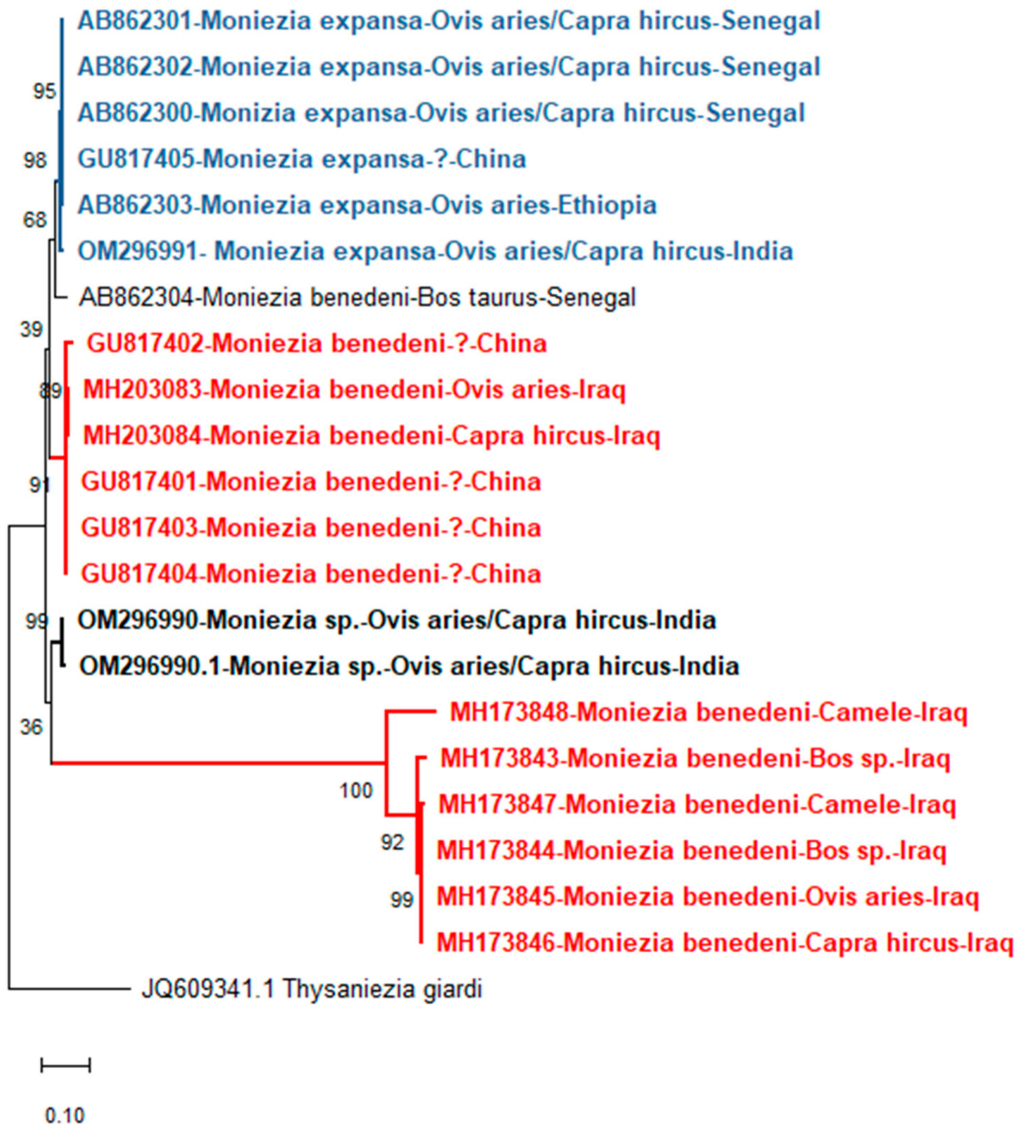

Figure 4: Phylogenetic tree obtained using the maximum likelihood method for the *SSU rDNA* gene of *Moniezia*. *?* = host not identified.

3.4.2. Haplotype Networks

The haplotype network for the *Cox1* gene shows that *M. expansa*, although quite close, forms two subgroups. *Moniezia* sp. are widely dispersed. Thus, certain *Moniezia* sp. from India (OQ134465.1) are grouped with *M. expansa*. *M. benedeni* form two subgroups, with some individuals quite distant from each other (Figure 5). The *SSU rDNA* haplotype network shows that all *M. expansa* form a group. As for *M. benedeni*, they are differentiated into two main groups, while *Moniezia* sp. form two groups, one of which is very close to *M. expansa* and the other very distant from both *M. expansa* and *M. benedeni* (Figure 6).

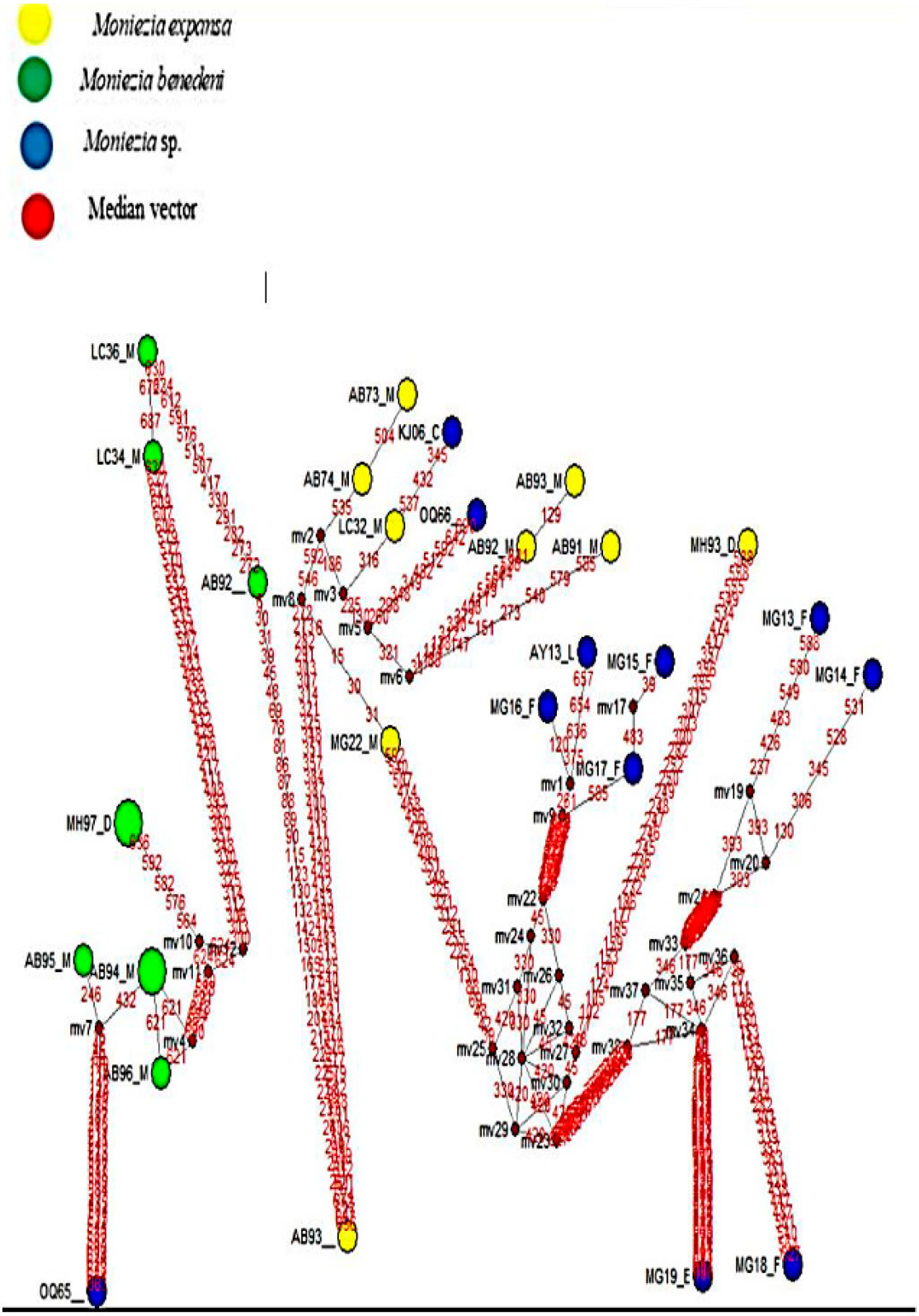

Figure 5: Haplotype network of 29 *Moniezia* individuals sampled based on *Cox1* gene sequences constructed with Network.

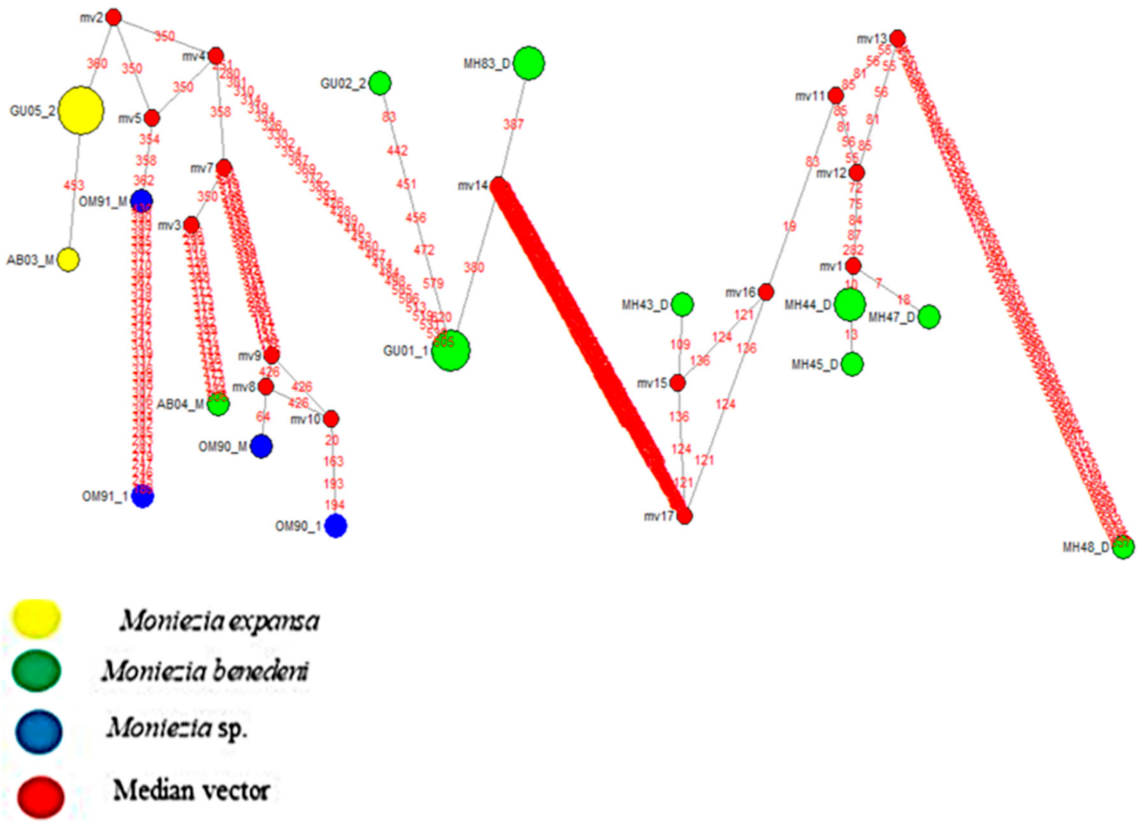

Figure 6: Haplotype network of 22 *Moniezia* individuals sampled, based on *SSU rDNA* gene sequences constructed with Network.

4. Discussion

Moniezia is a cosmopolitan genus of cestodes that parasitizes both wild and domestic ruminants [9]. These tapeworms inhabit the small intestine of their hosts, where they absorb nutrients and can cause significant health issues, including weight loss, reduced milk production, general weakness, and—in cases of heavy infestation—intestinal blockage that may lead to death [34]. To date, twelve species have been described across various hosts [15]. In domestic ruminants, however, only two morphological species—*M. expansa* and *M. benedeni*—have been traditionally recognized. A third species, *M. denticulata*, has recently been reported in small ruminants [9].

This study investigates the diversity, genetic differentiation, and population dynamics of *Moniezia* species infecting ruminants in Senegal. The analysis is based on 29 nucleotide sequences of the mitochondrial *Cox1* gene and 22 sequences of the nuclear *SSU rDNA* gene. Three species were identified: *M. expansa*, *M. benedeni*, and *Moniezia* sp. Genetic polymorphism and variability were higher in *M. benedeni* and *Moniezia* sp. than in *M. expansa*, consistent with previous findings suggesting cryptic species within *M. benedeni* [35,36].

Mitochondrial DNA analysis revealed high haplotypic and nucleotide diversity, with numerous distinct haplotypes identified. This diversity may be attributed to the following:

- Allopatric speciation, as the populations originate from geographically distant regions, limiting gene flow. This isolation likely facilitated independent evolutionary trajectories, resulting in distinct lineages. Our data show that haplotypes from the same country tend to cluster together, forming well-defined lineages (Figures 3 and 4).

- Reproductive strategy, since *Moniezia* species are hermaphroditic and capable of both cross- and self-fertilization [37]. These reproductive modes have different genetic consequences [37]. For instance, *Bulinus globosus* populations that reproduce via both methods exhibit greater genetic variability than *B. truncatus*, which predominantly self-fertilizes [38,39].

Nucleotide diversity (π) values were similar between *M. expansa* (0.048) and *M. benedeni* (0.043), while *Moniezia* sp. showed higher diversity (0.094), comparable to the overall population (0.090), indicating substantial intra-host and inter-regional variability [40].

Synonymous substitutions outnumbered non-synonymous ones, except in *M. benedeni*, where values were nearly equal (0.005 vs. 0.006), suggesting purifying selection and population stabilization.

Genetic structure analysis using Nei's genetic distance revealed clear differentiation: 12.7% between *M. expansa* and *M. benedeni*, and 12.8% between *M. benedeni* and *Moniezia* sp., supporting the existence of three distinct species. However, *Moniezia* sp. may represent a species complex, given that its intra-specific diversity (11.2%) is comparable to interspecific distances.

The global Fst value (0.3143, p = 0.0000) indicates significant genetic structuring, likely highly influenced by host species than geographic location [11].

Demographic tests (Fu's Fs) were non-significant across populations, suggesting demographic equilibrium or moderate expansion. This is supported by multimodal mismatch distributions, characteristic of stable populations.

Phylogenetic reconstruction via maximum likelihood grouped *M. expansa* from Senegal, Ethiopia, China, and Vietnam into a single subclade, while *M. benedeni* and *Moniezia* sp. each formed two distinct subclades. These patterns suggest host-specific lineages and limited parasite exchange between wild ruminants.

In contrast, genetic diversity among cestodes in domestic ruminants (sheep and goats) likely results from shared environments, such as pens and pastures [11,41].

The *Cox1* haplotype network revealed a *M. expansa* (AB099693) closely related to *M. benedeni*, and a *Moniezia* sp. (KJ576906) clustering with some *M. expansa* haplotypes, indicating cryptic species within the genus [35]. Three distinct groups were identified within *Moniezia* sp.

A unique *Moniezia* species may parasitize sheep, distinct from *M. expansa* and *M. benedeni*, as suggested by the Iraqi sequence (MH259793). Ref. [9] reported similar findings with *M. denticulata*, possibly resulting from host-switching events between small ruminants and cattle.

The goat-derived sample KJ576906, classified as *Moniezia* sp., and clustering with *M. expansa*, suggests that *Moniezia* sp. may include *M. expansa* and other yet-undescribed species.

For the *SSU rDNA* gene, polymorphism was highest in *M. benedeni* (378 sites), followed by *Moniezia* sp. (77 sites). *M. expansa* sequences were more conserved. These findings align with previous studies reporting greater diversity and cryptic species within *M. benedeni* [35,36].

Strong haplotypic and nucleotide diversity was observed in all populations except *M. expansa*, suggesting a stable population with a large effective size or admixture from previously isolated populations.

Nei's genetic distances for *SSU rDNA* were 0.429 between *M. expansa* and *M. benedeni*, and 0.456 between *M. benedeni* and *Moniezia* sp., confirming three distinct species. Individuals from *Moniezia* sp. were genetically closer to *M. expansa*. The high intra-population distance in *M. benedeni* (0.428) supports its classification as a species complex. In contrast, *M. expansa* showed zero intra-population distance, indicating genetic homogeneity.

The global Fst value (0.150, p = 0.0000) again confirms genetic structuring. Fu's Fs values were non-significant for *M. expansa* and *Moniezia* sp., supporting demographic stability. Mismatch distribution analysis also showed multimodal patterns, consistent with stable populations.

Maximum likelihood phylogenetic analysis of *SSU rDNA* grouped all *M. expansa* into one subclade, while *M. benedeni* formed two, reinforcing the presence of cryptic species within the genus.

The *SSU rDNA* haplotype network revealed clear differentiation between *M. expansa* and *M. benedeni*, and at least three phyletic groups within *M. benedeni*. *Moniezia* sp. may also comprise two distinct species.

Monieziosis causes significant economic losses in domestic ruminant farming. However, accurate morphological identification of the causative *Moniezia* species remains a major challenge. Molecular analyses are therefore essential to elucidate the diversity, structure, and dynamics of *Moniezia* populations.

This study demonstrates that *M. expansa* and *M. benedeni* are genetically diverse, with evidence of purifying selection and selective sweeps. Genetic structuring and demographic stability or moderate expansion were also observed.

Our findings show that *M. expansa* from Senegal are genetically closer to populations from Ethiopia, Vietnam, Iraq, and India (based on *Cox1*). Some *M. expansa* from Iraq and *Moniezia* sp. from India warrant further investigation. Both *Moniezia* sp. and *M. benedeni* appear to be species complexes. Morphological identification based solely on interproglottidal glands is insufficient; integrating molecular data with additional morphological criteria will improve species resolution.

5. Conclusion

Monieziosis represents a significant threat to the productivity of domestic ruminant farming, causing substantial economic losses worldwide. Despite its impact, accurate morphological identification of the *Moniezia* species responsible for this parasitic disease remains a persistent challenge in veterinary parasitology. Consequently, molecular approaches are essential to elucidate the diversity, population structure, and evolutionary dynamics of *Moniezia* species.

In this study, analyses of polymorphism and genetic variability based on the mitochondrial *Cox1* gene and nuclear *SSU rDNA* gene revealed high levels of genetic diversity within *M. expansa* and *M. benedeni*. The results also indicated clear genetic structuring across populations, consistent with demographic equilibrium or moderate population expansion.

Comparative analysis of *Moniezia* specimens from Senegal and other countries demonstrated that *M. expansa* from Senegal shares closer genetic affinity with populations from Ethiopia, Vietnam, Iraq, and India at the *Cox1* locus. However, certain *M. expansa* sequences from Iraq, as well as *Moniezia* sp. from India, warrant further investigation due to their genetic divergence. Moreover, both *Moniezia* sp. and *M. benedeni* exhibit considerable complexity, as previously suggested by multiple studies.

These findings underscore the limitations of traditional morphological identification, particularly those based solely on interproglottidal gland patterns and highlight the need for additional diagnostic criteria. Integrating molecular data with refined morphological markers will be crucial for achieving more accurate species delineation within the genus *Moniezia.*

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