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RESEARCH

Genetic variation and population structure of *Fasciola hepatica*: an in silico analysis

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Abstract

Fasciola hepatica is a trematode leading to heavy economic setbacks to the livestock sector globally. The population's genetic information and intimate kinship level are frequently assessed using analysis of mitochondrial DNA. In this analysis, we retrieved *cox*1 ($n = 247$) and *nad*1 ($n = 357$) sequences of *F. hepatica* from the NCBI GenBank database and aligned the sequences with the respective reference sequences using MEGA software. The median joining network was drawn using PopArt software while neutrality and diversity indices were estimated with the help of DnaSp software. Neighbor-joining phylogenetic tree was constructed using the MEGA software package. A total of 46 and 98 distinctive haplotypes were observed for *cox*1 and *nad*1 genes, respectively. Diversity indices indicated high haplotype and nucleotide diversities in both genes. Positive Tajima's *D* and Fu's Fs values were found for the entire population of both the genes under study. The *cox*1 and *nad*1 gene segments in this study showed high Tajima's *D* values, suggesting a low likelihood of future population growth. The Tajima's *D* value of the *nad*1 gene sequence is lower (2.14910) than that of the *cox*1 gene sequence (3.40314), which suggests that the former is growing at a slower rate. However, the region-wise analysis revealed that both the *cox*1 and *nad*1 genes showed deviation from neutrality suggesting a recent population expansion as a result of an excess of low-frequency polymorphism. Furthermore, the overall host-wise analysis showed positive and significant Tajima's *D* values for the *cox*1 and *nad*1 gene sequences. To the best of our knowledge, this is the first attempt to provide insights into genetic variations and population structure of *F. hepatica* at a global scale using *cox*1 and *nad*1 genes. Our findings suggest the existence of specific variants of *F. hepatica* in different parts of the world and provide information on the molecular ecology of *F. hepatica*. The results of this study also mark a critical development in upcoming epidemiological investigations on *F. hepatica* and will also contribute to understanding the global molecular epidemiology and population structure of *F. hepatica*.

Keywords *Fasciola hepatica* · *cox*1 · *nad*1 · Genetic variability

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Introduction

Trematodes of the genus *Fasciola* cause fasciolosis which is often known as liver fluke infection. The parasite *F. hepatica* infects animals with one of the widest regional distributions and causes significant losses to the global livestock industry (Charlier et al. 2020). It is listed as one of the most important neglected zoonotic diseases which affect both humans and domesticated animals (Mas-Coma et al. 2014). *Fasciola hepatica* causes a common foodborne illness in individuals that are linked to eating raw vegetables such as aquatic plants or alfalfa and drinking water that has been tainted with infective metacercaria (Cabada et al. 2018; Sabourin et al. 2018). Similar to humans, animals can get fasciolosis by consuming polluted food and water, which has a negative impact on their health and reduces animal output and, consequently, farm economic growth (Beesley et al. 2018).

Table 1 Accession numbers of *cox*1 and *nad*1 gene fragments of *F. hepatica* isolates used in the study

Origin	No. of isolates	*cox*1 Accession numbers	Origin	*nad*1 No. of isolates	Accession numbers
Algeria	51	LC485089-90-91-92-93-94-95-96-97-98-99, LC485100-01-02-03-04-05-06-07-08			LC436788-89-90-91-92-93-94-95-96-97-98-99
		MK212142-43-44-45-46-47-48 MT920965-66-67-68-69-70-71-72-73-74-75-76-77-78-79-80-81-82-83-84-85-86-87-88	Afghanistan	20	LC436800-01-02-03-04-05-06-07
Australia	2	AF216697 NC002546	Algeria	24	LC485065-66-67-68-69-70-71-72-73-74-75-76-77-78-79-80-81-82-83-84-85-86-87-88
		LC273025-26-27-28-29-30-31-32-33-34-35-36-37-38-39-40-41-42-43-44-45-46-47-48-49-50-51-52-53-54-55-56-57-58-59-60-61-62-63-64-65-66-67-68-69-70-71-72-73-74-75-76-77-78-79-80-81-82-83-84-85-86-87-88-89-90-91-92-93-94-95-96-97-98-99			
Ecuador	97	LC273100-01-02-03-04-05-06-07-08-09-10-11-12-13 MW867310-11-12-13-14-15-16-17	Argentina	14	MF959485-86-87-88/90-91-92-93-94-95-96-97-98-99
Egypt	3	MW217467 MW246125-26	Armenia	29	MG972375-76-77-78-79-80-81-82-83-84-85-86-87-88-89-90-91-92-93-94-95-96-97-98-99 MG972400-01-02-03
Iran	43	MG987175-76/78-79-80-81-82-83-84-85-86-87-88-89-90-91-92-93, MF788092/97-98-99, MF788101-02/05-06-07/13-14-15/21 OP600486-87-88-89, MG870562-63-64-66-67-68-69-70	Australia	2	AF216697 NC002546
Iraq	12	MN006833-34-35-36-37-38-39-40-41-42-43-44	Brazil	79	MK838688-89-90-91-92-93-94-95-96-97-98-99 MK838700-01-02-03-04-05-06-07-08-09-10-11-12-13-14-15-16-17-18-19-20-21-22-23-24-25-26-27-28-29-30-31-32-33-34-35-36-37-38-39-40-41-42-43-44-45-46-47-48-49-50-51-52-53-54-55-56-57-58-59-60-61-62-63-64-65-66

Table 1 (continued)

Origin	No. of isolates	*cox*1 Accession numbers	Origin	*nad*1 No. of isolates	Accession numbers
Italy	16	MT920989-90-91-92-93-94-95-96-97-98-99 MT921000-01-02-03-04	Ecuador	110	LC273114-15-16-17-18-19-20-21-22-23-24-25-26-27-28-29-30-31-32-33-34-35-36-37-38-39-40-41-42-43-44-45-46-47-48-49-50-51-52-53-54-55-56-57-58-59-60-61-62-63-64-65-66-67-68-69-70-71-72-73-74-75-76-77-78-79-80-81-82-83-84-85-86-87-88-89-90-91-92-93-94-95-96-97-98-99 LC273200-01-02 LC469792-93-94-95-96-97-98-99 LC469800-01-02-03-04 LC532273-74-75-76, MW867318-19-20-21
South Africa	6	OP265009/13-14-15-16-17	Iran	17	MF428469/71-72-73-74-75-76-77, MN527600-01-02-03-04-05-06, MN594514-15
Spain	17	GU112454-55-56-57, KF111586/90/93-94-95-96, KF111601-02/05/22/24-25/27	Japan	1	AP017707
			Spain	58	LC469053-54-55-56-57-58-59-60-61-62-63-64-65-66-67-68-69-70-71-72-73-74-75-76-77-78 KF111630-31-32-33/37-38/40-41-42/44-45/47/50-51-52-53-54-55-56-57-58-59-60/64/67-68/70-71/73-74-75/77
			Uruguay	3	MW867327-28-29

There are many zoonotic trematodiasis that have been estimated in the past to cause 665,352 disability-adjusted life years (DALYs) throughout the world (Fürst et al. 2012). Fasciolosis, a type of trematode infection that spreads through contaminated food, is found all around the world. Records show that *Fasciola* infection has been identified in 81 different countries (Caravedo and Cabada 2020). *Fasciola hepatica* is commonly present in tropical and subtropical regions such as those in the Middle East (Egypt and Iran), South America (Bolivia, Ecuador, and Peru), and Asia (Mas-Coma et al. 2009). Infection with *Fasciola* spp. increases the risk of co-infections, decreases production and fertility, and causes considerable morbidity and mortality in livestock. These factors add up to approximately €2.5 billion in annual economic losses worldwide (Mazeri et al. 2017). When animals consume infected encysted metacercariae that have erupted from the intermediate host and settled on the grass, they become infected. Following excystment in the gut, the parasite pierces the intestinal wall and migrates to the liver, where it causes two clinical phases of the disease to develop (Corrales et al. 2021). Acute fasciolosis sets in due to tissue destruction and hemorrhages brought on by the parasites tunneling and feeding activity in the liver tissue, as well as immunopathology brought on by the host's immune responses. In the chronic phase, the flukes move into the bile ducts, where they mature and lay eggs. Generally, the severity of infection is more in sheep than in cattle because they are less resistant to parasites. The most prevalent type of fasciolosis in sheep farms is subclinical and has minimal liver fluke loads, but it can still affect the wool quality and weight gain (Hayward et al. 2021) and make sheep more vulnerable to other diseases (Munita et al. 2019).

In addition to serving as a source of protein, livestock is seen as a direct source of revenue and employment (Mehmood et al. 2017). Thus, the food security and finance of the affected countries are threatened by this parasitosis (Webb and Cabada 2018). It is considered the primary contributor to financial losses in the cattle sector, particularly through liver damage, high morbidity and mortality, decreased output of meat and milk, and increased costs for anthelmintics and veterinary care (El-Tahawy et al. 2017; Fanke et al. 2017).

Table 2 Haplotypes of *cox*1 sequences of *F. hepatica* and accession numbers of isolates forming groups

Haplotype name	No. of isolates	Accession numbers
Hap01	64	LC485089-Algeria, LC485091-Algeria, LC485092-Algeria, LC485095-Algeria, LC485100-Algeria, LC485101-Algeria, LC485102-Algeria, LC273025-Ecuador, LC273026-Ecuador, LC273027-Ecuador, LC273030-Ecuador, LC273032-Ecuador, LC273033-Ecuador, LC273034-Ecuador, LC273036-Ecuador, LC273038-Ecuador, LC273039-Ecuador, LC273042-Ecuador, LC273049-Ecuador, LC273050-Ecuador, LC273051-Ecuador, LC273054-Ecuador, LC273060-Ecuador, LC273061-Ecuador, LC273062-Ecuador, LC273065-Ecuador, LC273066-Ecuador, LC273067-Ecuador, LC273068-Ecuador, LC273069-Ecuador, LC273070-Ecuador, LC273072-Ecuador, LC273074-Ecuador, LC273076-Ecuador, LC273077-Ecuador, LC273078-Ecuador, LC273079-Ecuador, LC273081-Ecuador, LC273082-Ecuador, LC273084-Ecuador, LC273085-Ecuador, LC273086-Ecuador, LC273087-Ecuador, LC273088-Ecuador, LC273089-Ecuador, LC273090-Ecuador, LC273091-Ecuador, LC273092-Ecuador, LC273093-Ecuador, LC273101-Ecuador, LC273102-Ecuador, LC273103-Ecuador, LC273104-Ecuador, LC273105-Ecuador, LC273107-Ecuador, LC273108-Ecuador, LC273109-Ecuador, LC273112-Ecuador, MW867312-Ecuador, MW867313-Ecuador, MW867315-Ecuador, MG987190-Iran, OP600487-Iran, MG870566-Iran
Hap02	30	LC485090-Algeria, LC273028-Ecuador, LC273029-Ecuador, LC273031-Ecuador, LC273035-Ecuador, LC273037-Ecuador, LC273040-Ecuador, LC273041-Ecuador, LC273043-Ecuador, LC273044-Ecuador, LC273045-Ecuador, LC273047-Ecuador, LC273048-Ecuador, LC273059-Ecuador, LC273063-Ecuador, LC273064-Ecuador, LC273071-Ecuador, LC273073-Ecuador, LC273075-Ecuador, LC273080-Ecuador, LC273094-Ecuador, LC273095-Ecuador, LC273096-Ecuador, LC273098-Ecuador, LC273099-Ecuador, LC273106-Ecuador, MW867311-Ecuador, MW867314-Ecuador, MW867316-Ecuador, MW867317-Ecuador
Hap03	1	LC485093-Algeria
Hap04	1	LC485094-Algeria
Hap05	1	LC485096-Algeria
Hap06	1	LC485097-Algeria
Hap07	1	LC485098-Algeria
Hap08	1	LC485099-Algeria
Hap09	1	LC485103-Algeria
Hap10	1	LC485104-Algeria
Hap11	1	LC485105-Algeria
Hap12	1	LC485106-Algeria
Hap13	5	LC485107-Algeria, MF788099-Iran, OP600486-Iran, OP600489-Iran, MG870562-Iran
Hap14	29	LC485108-Algeria, LC273055-Ecuador, LC273058-Ecuador, MW246125-Egypt, MG987178-Iran, MG987179-Iran, MG987180-Iran, MG987184-Iran, MG987191-Iran, MF788092-Iran, MF788097-Iran, MF788098-Iran, MF788101-Iran, MF788102-Iran, MF788106-Iran, MF788113-Iran, MF788114-Iran, MF788115-Iran, OP600488-Iran, MG870563-Iran, MG870567-Iran, MG870568-Iran, MG870569-Iran, MG987176-Iran, MG987183-Iran, MG987186-Iran, MG987187-Iran, MG987188-Iran, MG987189-Iran
Hap15	45	MK212142-Algeria, MK212144-Algeria, MK212147-Algeria, MT920966-Algeria, MT920967-Algeria, MT920969-Algeria, MT920970-Algeria, MT920971-Algeria, MT920972-Algeria, MT920973-Algeria, MT920974-Algeria, MT920975-Algeria, MT920976-Algeria, MT920977-Algeria, MT920978-Algeria, MT920979-Algeria, MT920980-Algeria, MT920981-Algeria, MT920982-Algeria, MT920983-Algeria, MT920985-Algeria, MT920986-Algeria, MT920984-Algeria, MT920987-Algeria, MT920988-Algeria, MN006843-Iraq, MT920989-Italy, MT920990-Italy, MT920991-Italy, MT920994-Italy, MT920996-Italy, MT920997-Italy, MT920998-Italy, MT920999-Italy, MT921000-Italy, MT921001-Italy, MT921002-Italy, MT921003-Italy, MT921004-Italy, OP265009-SouthAfrica, OP265014-SouthAfrica, OP265016-SouthAfrica, OP265017-SouthAfrica, KF111590-Spain, KF111602-Spain
Hap16	12	MK212143-Algeria, MN006834-Iraq, MN006835-Iraq, MN006836-Iraq, MN006837-Iraq, MN006838-Iraq, KF111586-Spain, KF111593-Spain, KF111594-Spain, KF111596-Spain, KF111601-Spain, KF111605-Spain
Hap17	1	MK212145-Algeria
Hap18	1	MK212146-Algeria
Hap19	1	MK212148-Algeria
Hap20	2	MT920965-Algeria, MT920968-Algeria
Hap21	2	AF216697-Australia, NC002546-Australia

Table 2 (continued)

Haplotype name	No. of isolates	Accession numbers
Hap22	1	LC273046-Ecuador
Hap23	9	LC273052-Ecuador, LC273056-Ecuador, LC273057-Ecuador, LC273097-Ecuador, LC273100-Ecuador, LC273110-Ecuador, LC273111-Ecuador, LC273113-Ecuador, MW867310-Ecuador
Hap24	1	LC273053-Ecuador
Hap25	1	LC273083-Ecuador
Hap26	2	MW217467-Egypt, MW246126-Egypt
Hap27	1	MG987185-Iran
Hap28	1	MG987192-Iran
Hap29	1	MF788105-Iran
Hap30	1	MF788107-Iran
Hap31	1	MF788121-Iran
Hap32	2	MG870564-Iran, MG870570-Iran
Hap33	1	MG987175-Iran
Hap34	2	MG987181-Iran, MG987182-Iran
Hap35	1	MG987193-Iran
Hap36	6	MN006833-Iraq, MT920992-Italy, MT920995-Italy, OP265013-SouthAfrica, OP265015-SouthAfrica, KF111595-Spain
Hap37	1	MN006839-Iraq
Hap38	2	MN006840-Iraq, KF111625-Spain
Hap39	2	MN006841-Iraq, KF111622-Spain
Hap40	1	MN006842-Iraq
Hap41	1	MN006844-Iraq
Hap42	1	MT920993-Italy
Hap43	3	GU112454-Spain, GU112455-Spain, GU112456-Spain
Hap44	1	GU112457-Spain
Hap45	1	KF111624-Spain
Hap46	1	KF111627-Spain

The occurrence and spread of the disease are also being impacted by climate change (Mas-Coma et al. 2019) and the trading in live animals is accelerating the spread of novel species to new areas as well as encouraging the development of hybrid forms (Agatsuma et al. 2000). *Fasciola* distribution causes controversy over species identification in several countries where morphological criteria are applied in research investigations. Presently, the GenBank database has a significant number of incomplete *nad*1 and *cox*1 gene sequences of *Fasciola* species isolated from different host species (Reaghi et al. 2016). Indicators like genetic diversity and population enable us to identify parasite adaptation and fitness in specific habitats (Rouhani et al. 2017).

Molecular studies play a significant role in the detection of dissemination of *Fasciola hepatica* due to host spectrum and ecological diversity (Thang et al. 2019). Diversity indices advocate that using the main population in defined ecosystems assists in better comprehending the biological perspectives and forecasting antigenic and phenotypic differences (Ai et al. 2011).

The genetic diversity of the *F. hepatica* population is also influenced by the intermediate hosts where clonal expansion occurs (Beesley et al. 2017). The potential of the intermediate host to spread metacercariae was confirmed by Vilas et al. (2012). Furthermore, the same multilocus genotype was found to be shared among cattle and sheep. The non-significant difference in genetic diversity of *F. hepatica* isolates from domesticated animals was observed by Beesley et al. (2017); however, a wide spectrum of wild animals being infected by *F. hepatica* is supposed to be responsible for maintaining genetic diversity. The available literature indicates a high genetic diversity level in *F. hepatica* populations, which could facilitate adaptation to environmental selection pressure through clonal reproduction in snails, allowing for the rapid spread of resistant populations (Elliott et al. 2014).

Table 3 The haplotypes of *nad*1 sequences of *F. hepatica* and accession numbers of isolates forming groups

Haplotype name	No. of isolates	Accession numbers
Hap01	8	LC436788-Afghanistan, LC436789-Afghanistan, LC436794-Afghanistan, LC436795-Afghanistan, LC436804-Afghanistan, MG972378-Armenia, MF428469-Iran, MN594514-Iran
Hap02	2	LC436790-Afghanistan, MG972398-Armenia
Hap03	1	LC436791-Afghanistan
Hap04	1	LC436792-Afghanistan
Hap05	3	LC436793-Afghanistan, MG972377-Armenia, MF428476-Iran
Hap06	24	LC436796-Afghanistan, LC436803-Afghanistan, LC436805-Afghanistan, LC436806-Afghanistan, LC436807-Afghanistan, LC485088-Algeria, MG972400-Armenia, MG972401-Armenia, MG972402-Armenia, MG972375-Armenia, MG972382-Armenia, MG972383-Armenia, MG972392-Armenia, MG972394-Armenia, MG972397-Armenia, AF216697-Australia, NC002546-Australia, MF428477-Iran, MN527600-Iran, MN527601-Iran, MN527602-Iran, MN527606-Iran, MN594515-Iran, LC469078-Spain
Hap07	1	LC436797-Afghanistan
Hap08	1	LC436798-Afghanistan
Hap09	1	LC436799-Afghanistan
Hap10	1	LC436800-Afghanistan
Hap11	113	LC436801-Afghanistan, LC485065-Algeria, LC485066-Algeria, LC485070-Algeria, LC485071-Algeria, LC485072-Algeria, LC485076-Algeria, LC485077-Algeria, LC485081-Algeria, LC485084-Algeria, LC485085-Algeria, LC485086-Algeria, MF959488-Argentina, MF959490-Argentina, MF959491-Argentina, MF959492-Argentina, MF959493-Argentina, MF959494-Argentina, MF959495-Argentina, MF959498-Argentina, MG972376-Armenia, MG972380-Armenia, MG972384-Armenia, LC273114-Ecuador, LC273115-Ecuador, LC273116-Ecuador, LC273117-Ecuador, LC273118-Ecuador, LC273119-Ecuador, LC273120-Ecuador, LC273121-Ecuador, LC273122-Ecuador, LC273123-Ecuador, LC273124-Ecuador, LC273125-Ecuador, LC273126-Ecuador, LC273127-Ecuador, LC273128-Ecuador, LC273129-Ecuador, LC273130-Ecuador, LC273131-Ecuador, LC273132-Ecuador, LC273133-Ecuador, LC273135-Ecuador, LC273136-Ecuador, LC273137-Ecuador, LC273138-Ecuador, LC273139-Ecuador, LC273140-Ecuador, LC273141-Ecuador, LC273142-Ecuador, LC273143-Ecuador, LC273148-Ecuador, LC273149-Ecuador, LC273150-Ecuador, LC273151-Ecuador, LC273152-Ecuador, LC273153-Ecuador, LC273154-Ecuador, LC273155-Ecuador, LC273156-Ecuador, LC273157-Ecuador, LC273158-Ecuador, LC273159-Ecuador, LC273160-Ecuador, LC273161-Ecuador, LC273162-Ecuador, LC273164-Ecuador, LC273165-Ecuador, LC273166-Ecuador, LC273167-Ecuador, LC273168-Ecuador, LC273169-Ecuador, LC273170-Ecuador, LC273171-Ecuador, LC273172-Ecuador, LC273173-Ecuador, LC273174-Ecuador, LC273175-Ecuador, LC273176-Ecuador, LC273177-Ecuador, LC273178-Ecuador, LC273179-Ecuador, LC273180-Ecuador, LC273181-Ecuador, LC273182-Ecuador, LC273183-Ecuador, LC273184-Ecuador, LC273187-Ecuador, LC273191-Ecuador, LC273192-Ecuador, LC273193-Ecuador, LC273194-Ecuador, LC273196-Ecuador, LC273197-Ecuador, LC273198-Ecuador, LC273201-Ecuador, LC469792-Ecuador, LC469793-Ecuador, LC469795-Ecuador, LC469796-Ecuador, LC469802-Ecuador, MW867318-Ecuador, MW867319-Ecuador, MW867321-Ecuador, AP017707-Japan, LC469053-Spain, LC469054-Spain, LC469063-Spain, LC469073Spain KF111660-Spain, MW867327-Uruguay, MW867328-Uruguay
Hap12	1	LC436802-Afghanistan
Hap13	33	LC485067-Algeria, LC273145-Ecuador, LC273146-Ecuador, LC273186-Ecuador, LC273189-Ecuador, LC273199-Ecuador, LC273200-Ecuador, LC273202-Ecuador, LC469794-Ecuador, LC532276-Ecuador, MW867320-Ecuador, MF428475-Iran, MF428472-Iran, MN527603-Iran, MN527604-Iran LC469076-Spain, LC469077-Spain, KF111631-Spain, KF111633-Spain, KF111644-Spain, KF111647-Spain, KF111650-Spain, KF111651-Spain, KF111652-Spain, KF111653-Spain, KF111658-Spain, KF111664-Spain, KF111667-Spain, KF111668-Spain, KF111670-Spain, KF111674-Spain, KF111675-Spain, MW867329-Uruguay
Hap14	2	LC485068-Algeria, LC469066-Spain
Hap15	2	LC485069-Algeria, LC469070-Spain
Hap16	1	LC485073-Algeria
Hap17	3	LC485074-Algeria, LC273185-Ecuador, LC273188-Ecuador
Hap18	1	LC485075-Algeria
Hap19	1	LC485078-Algeria
Hap20	1	LC485079-Algeria
Hap21	3	LC485080-Algeria, LC532273-Ecuador, LC469056-Spain
Hap22	1	LC485082-Algeria
Hap23	1	LC485083-Algeria

Table 3 (continued)

Haplotype name	No. of isolates	Accession numbers
Hap24	1	LC485087-Algeria
Hap25	4	MF959485-Argentina, MF959486-Argentina, MF959487-Argentina, MF959497-Argentina
Hap26	2	MF959496-Argentina, MF959499-Argentina
Hap27	1	MG972399-Armenia
Hap28	2	MG972403-Armenia, MG972395-Armenia
Hap29	1	MG972379-Armenia
Hap30	1	MG972381-Armenia
Hap31	1	MG972385-Armenia
Hap32	1	MG972386-Armenia
Hap33	1	MG972387-Armenia
Hap34	1	MG972388-Armenia
Hap35	1	MG972389-Armenia
Hap36	1	MG972390-Armenia
Hap37	1	MG972391-Armenia
Hap38	1	MG972393-Armenia
Hap39	1	MG972396-Armenia
Hap40	59	MK838688-Brazil, MK838689-Brazil, MK838690-Brazil, MK838691-Brazil, MK838692-Brazil, MK838698-Brazil, MK838699-Brazil, MK838700-Brazil, MK838702-Brazil, MK838704-Brazil, MK838705-Brazil, MK838706-Brazil, MK838709-Brazil, MK838714-Brazil, MK838716-Brazil, MK838718-Brazil, MK838719-Brazil, MK838720-Brazil, MK838721-Brazil, MK838722-Brazil, MK838723-Brazil, MK838724-Brazil, MK838725-Brazil, MK838726-Brazil, MK838727-Brazil, MK838728-Brazil, MK838729-Brazil, MK838730-Brazil, MK838731-Brazil, MK838732-Brazil, MK838733-Brazil, MK838734-Brazil, MK838735-Brazil, MK838736-Brazil, MK838737-Brazil, MK838738-Brazil, MK838739-Brazil, MK838740-Brazil, MK838741-Brazil, MK838742-Brazil, MK838743-Brazil, MK838744-Brazil, MK838745-Brazil, MK838746-Brazil, MK838749-Brazil, MK838750-Brazil, MK838751-Brazil, MK838752-Brazil, MK838753-Brazil, MK838754-Brazil, MK838755-Brazil, MK838757-Brazil, MK838759-Brazil, MK838760-Brazil, MK838761-Brazil, MK838762-Brazil, MK838763-Brazil, MK838764-Brazil, MK838765-Brazil
Hap41	4	MK838693-Brazil, MK838710-Brazil, MK838712-Brazil, MK838713-Brazil
Hap42	1	MK838694-Brazil
Hap43	1	MK838695-Brazil
Hap44	1	MK838696-Brazil
Hap45	1	MK838697-Brazil
Hap46	1	MK838701-Brazil
Hap47	1	MK838703-Brazil
Hap48	1	MK838707-Brazil
Hap49	1	MK838708-Brazil
Hap50	1	MK838711-Brazil
Hap51	1	MK838715-Brazil
Hap52	1	MK838717-Brazil
Hap53	1	MK838747-Brazil
Hap54	1	MK838748-Brazil
Hap55	1	MK838756-Brazil
Hap56	2	MK838758-Brazil, MK838766-Brazil
Hap57	3	LC273134-Ecuador, LC273195-Ecuador, LC532275-Ecuador
Hap58	2	LC273144-Ecuador, LC273147-Ecuador
Hap59	1	LC273163-Ecuador
Hap60	1	LC273190-Ecuador
Hap61	2	LC469797-Ecuador, LC469075-Spain

Table 3 (continued)

Haplotype name	No. of isolates	Accession numbers
Hap62	2	LC469798-Ecuador, LC469064-Spain
Hap63	1	LC469799-Ecuador
Hap64	1	LC469800-Ecuador
Hap65	1	LC469801-Ecuador
Hap66	1	LC469803-Ecuador
Hap67	1	LC469804-Ecuador
Hap68	1	LC532274-Ecuador
Hap69	1	MF428473-Iran
Hap70	1	MF428471-Iran
Hap71	1	MF428474-Iran
Hap72	1	MN527605-Iran
Hap73	1	LC469055-Spain
Hap74	1	LC469058-Spain
Hap75	1	LC469060-Spain
Hap76	1	LC469061-Spain
Hap77	1	LC469062-Spain
Hap78	1	LC469065-Spain
Hap79	1	LC469068-Spain
Hap80	1	LC469069-Spain
Hap81	1	LC469072-Spain
Hap82	1	LC469057-Spain
Hap83	1	LC469059-Spain
Hap84	1	LC469067-Spain
Hap85	1	LC469071-Spain
Hap86	1	LC469074-Spain
Hap87	2	KF111630-Spain, KF111637-Spain
Hap88	1	KF111632-Spain
Hap89	1	KF111638-Spain
Hap90	1	KF111640-Spain
Hap91	1	KF111641-Spain
Hap92	1	KF111642-Spain
Hap93	1	KF111645-Spain
Hap94	4	KF111654-Spain, KF111656-Spain, KF111657-Spain, KF111677-Spain
Hap95	1	KF111655-Spain
Hap96	1	KF111659-Spain
Hap97	1	KF111671-Spain
Hap98	1	KF111673-Spain

Molecular studies that employ both nuclear and mitochondrial DNA have identified diverse fluke populations across the globe. The nuclear DNA markers, ribosomal internal transcribed spacer (ITS), phosphoenolpyruvate carboxykinase (pepck), DNA polymerase delta (pold), cytochrome *c* oxidase I (*cox*1), and NADH dehydrogenase (*nad*1) genes are some of the markers that can be used for genetic characterization of *Fasciola* spp., as described by Sarkari et al. (2017) and Chougar et al. (2019).

Mitochondrial DNA (mt-DNA), has been used as a marker for population diversity. Mitochondrial *cox*1 and *nad*1 genes are frequently used as DNA markers to investigate the genetic variability of *Fasciola* spp. These markers are of high importance to study biogeography and population structure of *F. hepatica* (Itagaki et al. 2005). Furthermore, microsatellite DNA simple sequence repeat markers have also been studied to reveal the population structure of various parasites (Yin et al.

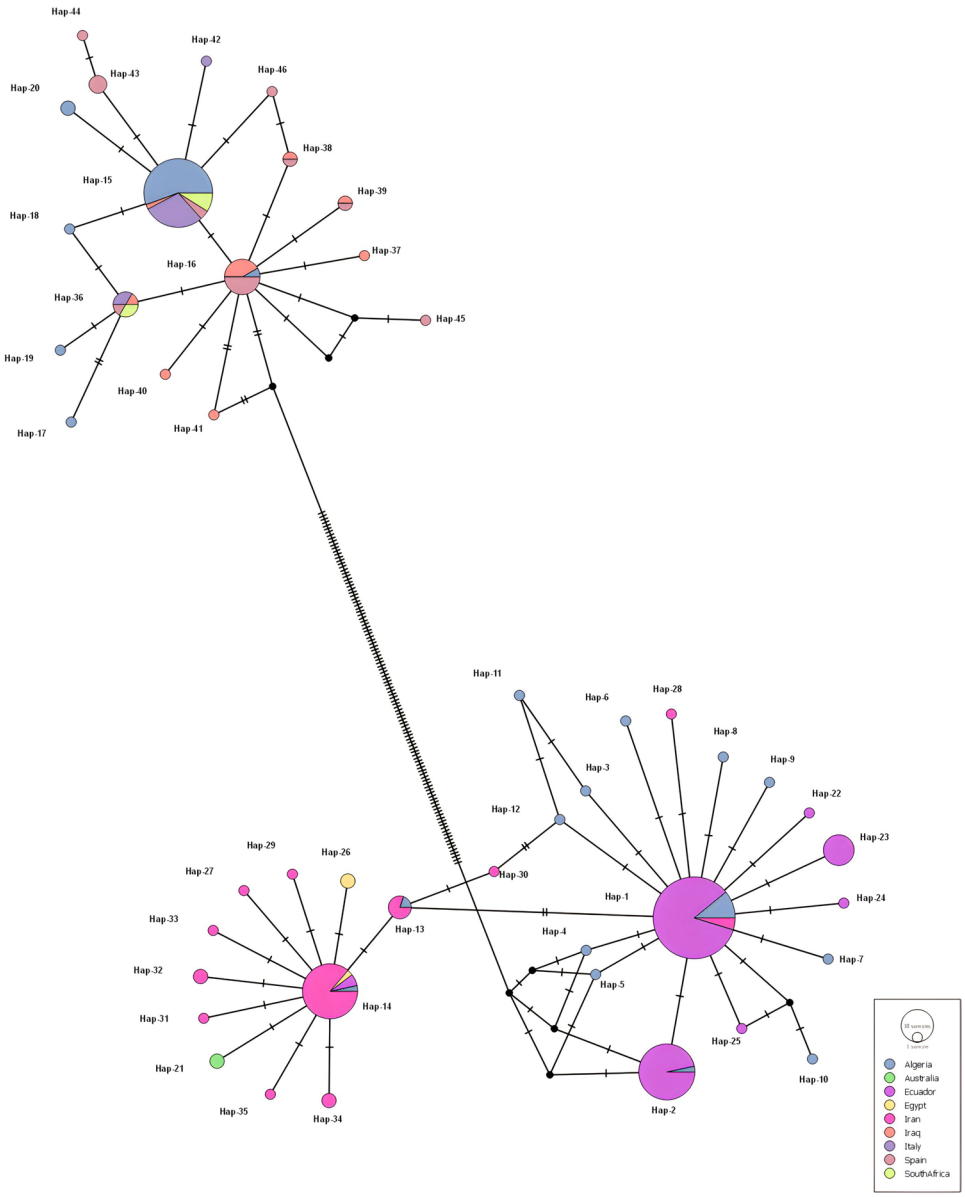

Fig. 1 Appearance of *cox*1 (387 bp) haplotypes *F. hepatica* sequences. The number of mutations that distinguish haplotypes is indicated by screening marks. The geographical distribution of haplotypes is shown in different colors. The size of the circles is related to the haplotype frequency

2016; Moendeg et al. 2017). The genetic information of the population and the level of intimate kinship are frequently assessed using analysis of mt-DNA sequences because of the absence of recombination, maternal inheritance, conserved structure, higher mutation rate and a relatively higher evolutionary rate (Jia et al. 2012). To identify genetic variations within *Fasciola* species, incomplete sequences of *cox*1 and *nad*1 genes have been effectively employed (Bowles and McManus 1993). To understand the population dynamics, it is essential to assess the genetic diversity in *F. hepatica* isolates globally. This study analyzed the *cox*1 and *nad*1 gene sequences available in the GenBank repository to describe genetic variation, population structure, and phylogeny of *F. hepatica*.

Methods

Data Collection

A total of 604 gene sequences of *F. hepatica* were retrieved and a dataset was produced after selecting the *cox*1 ($n = 247$) and *nad*1 ($n = 357$) genes, submitted to the National Center for Biotechnology Information, USA (NCBI), database until 9 February 2023.

Alignment and Phylogenetic Analysis

By using Molecular Evolutionary Genetics Analysis Version 11 (MEGA 11), all gene sequences were

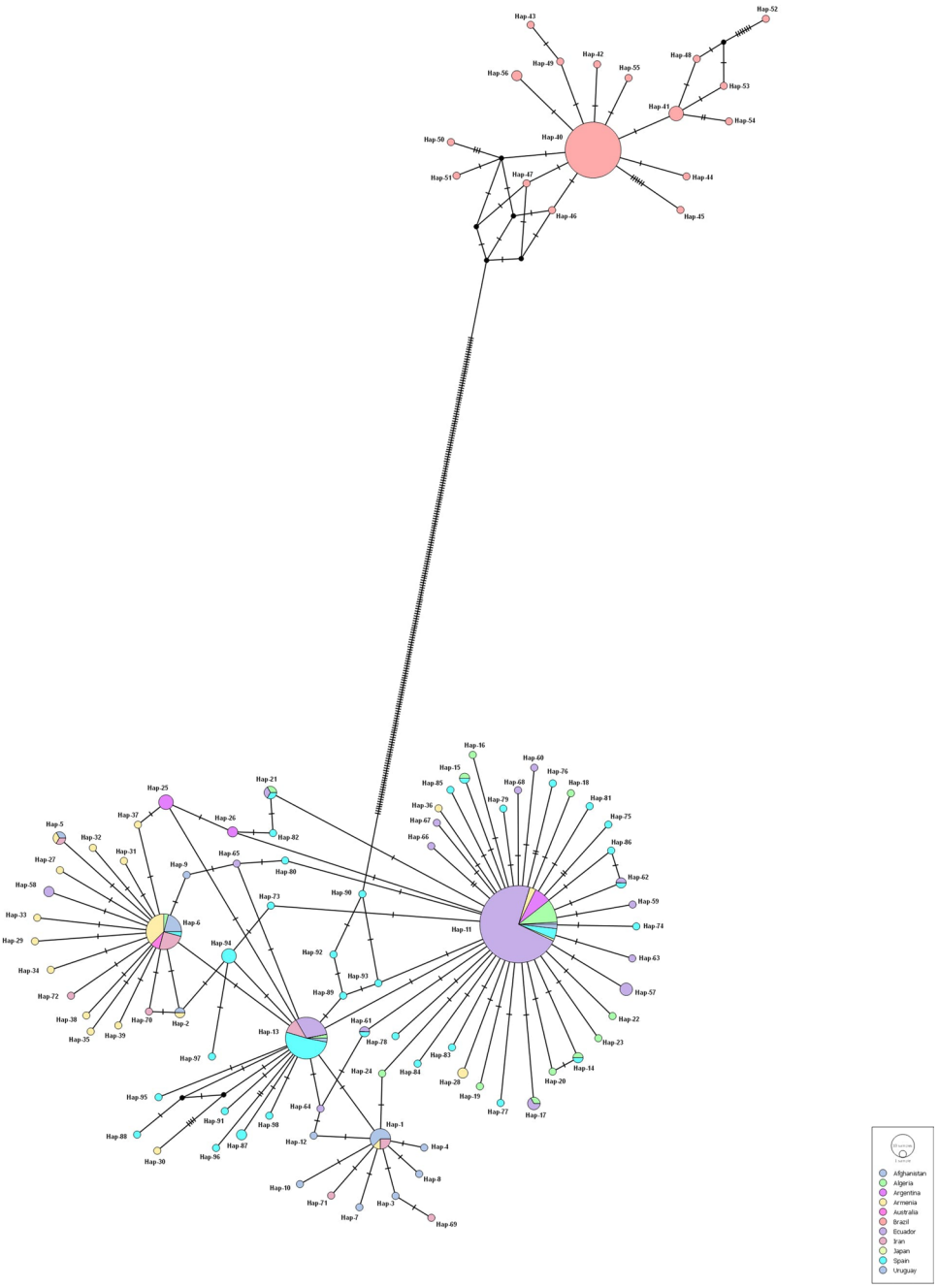

Fig. 2 Appearance of *nad*1 (689 bp) haplotypes *F. hepatica* sequences. The number of mutations that distinguish haplotypes is indicated by screening marks. The geographical distribution of haplotypes is shown in different colors. The size of the circles is related to the haplotype frequency.

assembled into it in FASTA format. The reference sequences *cox*1 (accession no. LC273025) and *nad*1 (accession no. MK838688) were used to align all of the sequences after they had been cut off at both ends. Short gene sequences were eliminated leaving 604 gene sequences of 387 bp *cox*1 ($n = 247$) and 689 bp *nad*1 ($n = 357$) for bioinformatic analysis. Phylogenetic trees were created from the sequences of both gene regions using the neighbor-joining (NJ) model and the Jukes–Cantor nucleotide distance measure. The 1000 bootstrap replicates were used to obtain statistical support for the branch specificity. To determine the strength of the relationships, the sequence of *Schistosoma japonicum* was used as an outgroup.

Haplotype analysis and networking

The sequences in FASTA format were examined during the haplotype analysis by using the tool DnaSP 6 (Rozas et al. 2017). Calculations were made to estimate the genetic composition of both the genes using the haplotype and nucleotide change values, nucleotide and haplotype numbers,

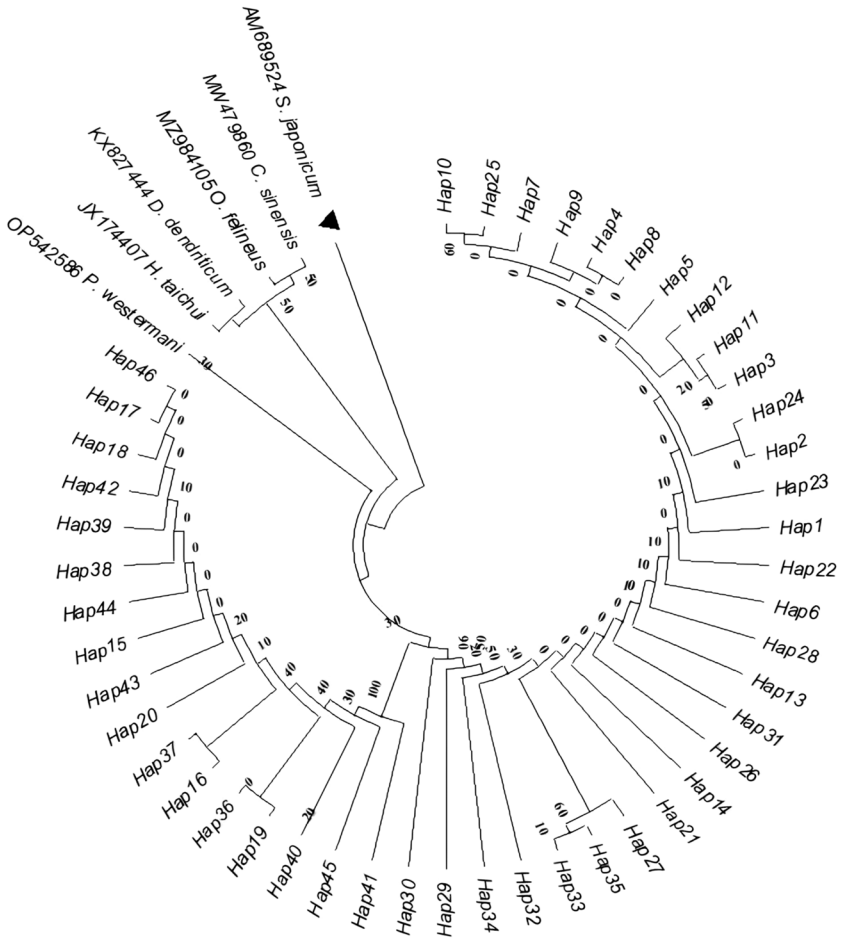

Fig. 3 Phylogenetic tree view of *F. hepatica* sequences using *cox*1 gene (387 bp)

and the neutrality indices. For a visual depiction of the associations between haplotypes, the sequences were converted to Nexus format (Maddison et al. 1997) and a haplotype network was created using the PopArt (Population Analysis with Reticulate Trees) application (Leigh and Bryant 2015).

Results

A total of 604 gene sequences from *F. hepatica* isolates were analyzed in this study, comprising 247 *cox*1 sequences obtained from 9 countries and 357 *nad*1 sequences obtained from 11 countries (as shown in Table 1). All of these sequences were sourced from the NCBI database.

Polymorphism and haplotype analysis

Mutations were detected at 154 and 275 different points within the *cox*1 and *nad*1 gene sequences. Forty-six distinctive haplotypes were identified by the analysis of 247 *cox*1 gene sequences (Table 2). The dominating haplotype among these was Hap01, which had 64 genetic sequences. By evaluating 357 *nad*1 genetic sequences, 98 haplotypes were recognized (Table 3). Out of these, Hap11 was the dominant haplotype and consisted of 113 gene sequences.

Haplotype network

There were 46 haplotypes in the *cox*1 haplotype network (Fig. 1). The major haplotype in the network was Hap01, which contributed 25.91% (64/247) and was followed by Hap15 with 18.21% (45/247). A single distinct haplotype mainly composed 65.21% (30/46) of the total haplotype network. Individual haplotypes came from Algeria ($n = 13$), Iran ($n = 7$), Ecuador ($n = 3$), Iraq ($n = 3$), Spain ($n = 3$), and Italy ($n = 1$).

There were 98 haplotypes in the *nad*1 haplotype network (Fig. 2). The major haplotype in the network was Hap11, which contributed 31.65% (113/357) and was followed by

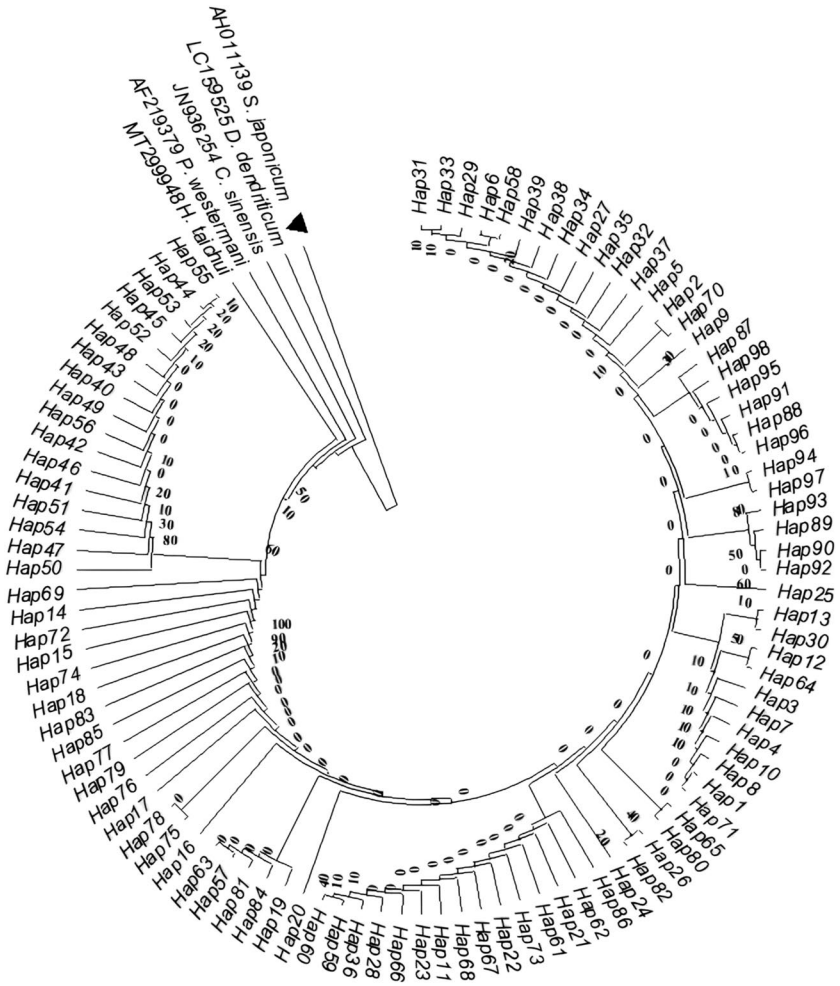

Fig. 4 Phylogenetic tree view of *F. hepatica* sequences using *nad*1 gene (689 bp)

Table 4 Diversity and neutrality indices obtained using nucleotide data of the *cox*1 (387 bp) and *nad*1 (689 bp) gene sequences of *F. hepatica*

Indices	*cox*1 (387 bp)	*nad*1 (689 bp)
No. of sequences	247	357
No. of mutations	154	275
Parsimony informative sites	125	224
No. of haplotypes	46	98
Haplotype diversity (Hd)	0.869±0.012	0.860±0.014
Nucleotide diversity (π)	0.17426±0.00761	0.21029±0.01457
Tajima's *D*	3.40314	2.14910
Fu's Fs	28.638	10.136
FLD	0.28279	−0.18109*
FLF	2.16290	1.23702

Hd haplotype diversity, *π* nucleotide diversity, *FLD* Fu and Li's *D* test statistic, *FLF* Fu and Li's *F* test statistic

Hap40 with 16.52% (59/357). A single distinct haplotype mainly composed 77.55% (76/98) of the total haplotype network. Individual haplotypes came from Spain ($n = 24$), Brazil ($n = 14$), Armenia ($n = 12$), Ecuador ($n = 8$), Afghanistan ($n = 7$), Algeria ($n = 7$), and Iran ($n = 4$).

Phylogenetic tree

The findings of the phylogenetic analysis were reliable with the haplotype network. The *cox*1 and *nad*1 gene sequences were aligned to create the phylogenetic tree as mentioned in Figs. 3 and 4, respectively. *Schistosoma japonicum* was added as outgroup in both phylogenetic trees. The evolutionary history was inferred using the neighbor-joining method (Saitou and Nei 1987). The optimal trees are shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches (Felsenstein 1985). The evolutionary distances were computed using the maximum composite likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. This analysis involved 52 and 103 nucleotide sequences for *cox*1 and *nad*1 trees, respectively. Codon positions included were 1st+2nd+3rd+Noncoding.

Table 5 Region-wise diversity and neutrality indices obtained using nucleotide data of the *cox*1 gene sequences of *F. hepatica* obtained from the NCBI GenBank database

Indices	Algeria	Australia	Ecuador	Egypt	Iran	Iraq	Italy	South Africa	Spain
No. of sequences	51	2	97	3	43	12	16	6	17
No. of mutations	146	0	10	1	12	9	3	2	11
Parsimony informative sites	130	0	6	0	5	0	2	2	4
No. of haplotypes	21	0	7	2	12	9	3	2	11
Haplotype diversity (Hd)	0.752	0	0.598	0.667	0.656	0.945	0.342	0.533	0.926
Nucleotide diversity (π)	0.19359	0	0.00246	0.00196	0.00375	0.00441	0.00159	0.00288	0.00647
Tajima's *D*	3.36173*	–	−1.45834	–	−1.73311	−1.96450*	−1.00180	1.03194	−1.06081
Fu's Fs	16.949	0	−1.838	0.201	−7.589	−7.495	−0.067	1.723	−6.219
FLD	1.45084*	–	−1.42544	–	−2.16280	−2.36231*	−0.03858	1.27971	−1.56644
FLF	2.59885*	–	−1.70555	–	−2.38309	−2.55641*	−0.33572	1.27479	−1.47395

* Indicates statistical significance with $p<0.05$. – Indicates that value could not be computed as four or more sequences are needed to compute Tajima's and Fu and Li's statistics

All ambiguous positions were removed for each sequence pair (pairwise deletion option). Evolutionary analyses were conducted in MEGA11 (Tamura et al. 2021).

Gene flow, diversity, and neutrality analysis

In Table 4, the diversity and neutrality indices are given for the *cox*1 and *nad*1 genes. To ascertain whether populations were under selection pressure, the values of Tajima's *D* and Fu's Fs were determined.

Diversity and neutrality indices for *F. hepatica* isolates from various geographical regions

To provide insights into the future population growth in each region, the sequences used in the study were further analyzed to compute diversity and neutrality indices. Diversity and neutrality indices of *cox*1 and *nad*1 genes are shown in Tables 5 and 6, respectively.

For *cox*1 gene sequences from Ecuador, Iran, Italy, and Spain, the negative but insignificant values of Tajima's *D* were observed while this parameter was statistically significant and negative for the Iraqian isolates. This indicated deviation from neutrality suggesting a recent population expansion as a result of an excess of low-frequency polymorphism. A positively significant value of Tajima's *D* was observed for the isolates from Algeria and the value was also positive but insignificant for the isolates from South Africa. Diversity indices were not calculated for the isolates from Australia and Egypt as four or more sequences are needed to compute Tajima's *D* value. Fu's Fs was negative for the entire population indicating an excess number of alleles, as would be expected during genetic hitchhiking or a recent population expansion except for the Algerian and South African isolates which were positive but insignificant.

Regarding *nad*1 gene sequences, the negatively significant values of Tajima's *D* were observed for the isolates from Algeria, Armenia, Brazil, Ecuador, and Spain. Like *cox*1 gene sequences, this also indicated deviation from neutrality suggesting a recent population expansion. Diversity indices were not calculated for the isolates from Australia and Uruguay as four or more sequences are needed to compute Tajima's *D* value. Fu's Fs was negative for the entire population indicating an excess number of alleles, except for the Argentinian isolates which were positive but insignificant. For *nad*1 gene analysis, Japan was excluded in indices calculations as only one sequence was included from Japan in this study.

Host-based diversity and neutrality indices for *F. hepatica* isolates

In order to provide a better comprehensive understanding of the genetic dynamics of the parasite in various hosts and its circulation in the environment, the sequences used in the study were further analyzed to compute diversity and neutrality indices based on their host origin. A total of 12 *cox*1 and 20 *nad*1 sequences available in the NCBI GenBank database were without any information about their host. Thus, only 235 *cox*1 and 337 *nad*1 gene sequences were included in the host-wise analysis of data. Diversity and neutrality indices of *cox*1 and *nad*1 genes are shown in Tables 7 and 8, respectively.

Table 6 Region-wise diversity and neutrality indices obtained using nucleotide data of the *nad*1 gene sequences of *F. hepatica* obtained from the NCBI GenBank database

Indices	Afghanistan	Algeria	Argentina	Armenia	Australia	Brazil	Ecuador	Iran	Spain	Uruguay
No. of sequences	20	24	14	29	2	79	110	17	58	3
No. of mutations	22	22	3	30	0	38	21	10	44	2
Parsimony informative sites	4	2	3	5	0	13	10	3	10	0
No. of haplotypes	20	22	4	21	0	22	20	9	43	3
Haplotype diversity (Hd)	1.000	0.989	0.747	0.943	0	0.735	0.716	0.860	0.972	1.000
Nucleotide diversity (π)	0.00702	0.00445	0.00240	0.00647	0	0.00374	0.00263	0.00488	0.00633	0.00260
Tajima's *D*	−1.60711	−2.25351*	0.93268	−2.05549*	–	−2.39862*	−1.98317*	−0.87638	−2.22697*	–
Fu's Fs	−23.416	−30.101	0.037	−16.897	0	−15.294	−16.038	−3.423	−57.955	−1.216
FLD	−2.81488*	−3.66033*	1.07042	−3.72302*	–	−4.43723*	−3.07045*	−1.73496	−4.99300*	–
FLF	−2.85942*	−3.77690*	1.17727	−3.75144*	–	−4.36909*	−3.17836*	−1.72396	−4.73515*	–

*Indicates statistical significance with $p < 0.05$. – Indicates that value could not be computed as four or more sequences are needed to compute Tajima's and Fu and Li's statistics

Regarding the *cox*1 gene sequences, the values of Tajima's *D* were negative but insignificant for the isolates of goat and human origin. Overall, positive and significant Tajima's *D* values were observed. A similar pattern of Fu's Fs values was observed.

Regarding the *nad*1 gene, the negative and statistically significant value of Tajima's *D* indicated events of recent population expansion while this value was positive for the isolates of cattle origin. Fu's Fs was negative for the isolates of sheep origin while the overall value was insignificant and positive.

The *cox*1 and *nad*1 gene sequences were aligned to create the phylogenetic tree (Figs. 5 and 6) to describe host-wise diversity. *Schistosoma japonicum* was added as an outgroup in both phylogenetic trees. Representative haplotypes were included to construct the phylogenetic trees. For *nad*1, 52 cattle and 60 sheep isolates were included out of a dataset of 337 sequences while for the *cox*1 gene, 35 cattle, 15 sheep, 5 goat, and 4 human samples were included out of a dataset of 235 sequences.

Discussion

Infectious diseases including parasitic infestations are important health problems in both animals and human beings (Mo'awad et al. 2022; Mahmoud et al. 2022). Parasites, in addition to causing diseases in animals, indirectly affect the economics of the farmer by decreasing the productivity potential of animals (Mahmood et al. 2022; Alberfkani et al. 2022). Due to the growing infection risks, fasciolosis, a recurrent ignored tropical disease, is becoming a significant issue for both food quality and human safety. The current study offers significant findings about the genetic analysis and global diversity of *F. hepatica*.

High levels of rainfall, the optimal combination of temperature and humidity, public eating habits, ecological factors of *Fasciola* transmission, free ruminant grazing, and the presence of an intermediate host favor the propagation of fasciolosis in an area (Akhlaghi et al. 2017). It is widely known that maximum genetic flow, which increases the actual population size across a range of geographical zones where the features associated with heterogeneity may be prevalent, can frequently result in the genetic variation of parasites. Due to the variety of hosts and ecological circumstances, molecular epidemiology investigations are essential for identifying *Fasciola* dissemination (Thang et al. 2019).

Table 7 Host-wise diversity and neutrality indices obtained using nucleotide data of the *cox*1 gene sequences of *F. hepatica* obtained from the NCBI GenBank database

Indices	Cattle	Sheep	Goat	Human	Overall
No. of sequences	184	32	13	6	235
No. of mutations	152	136	4	5	154
Parsimony informative sites	126	124	1	1	124
No. of haplotypes	35	15	5	4	44
Haplotype diversity (Hd)	0.824	0.917	0.538	0.867	0.862
Nucleotide diversity (π)	0.18334	0.20152	0.00234	0.00567	0.17711
Tajima's *D*	3.74943*	3.30514*	−1.43714	−0.65543	3.46768*
Fu's Fs	36.964	14.174	−2.535	−0.561	30.226
FLD	0.57884	1.45494*	−1.35316	−0.79148	0.18774
FLF	2.48332*	2.47014*	−1.56136	−0.81207	2.13253*

* Indicates statistical significance with $p < 0.05$

Table 8 Host-wise diversity and neutrality indices obtained using nucleotide data of the *nad*1 gene sequences of *F. hepatica* obtained from the NCBI GenBank database

Indices	Cattle	Sheep	Overall
No. of sequences	236	101	337
No. of mutations	250	59	270
Parsimony informative sites	217	15	223
No. of haplotypes	52	60	92
Haplotype diversity (Hd)	0.767	0.972	0.856
Nucleotide diversity (π)	0.26927	0.00692	0.21882
Tajima's *D*	3.90568*	−2.30805*	2.41940*
Fu's Fs	50.340	−85.186	13.441
FLD	1.02197	−6.34956*	0.10673
FLF	2.92724*	−5.62365*	1.55707

High variability has also been observed in other research that has used haplotype diversity to investigate *F. hepatica*. For instance, research employing the *nad*1 gene identified 24 haplotypes from 79 Brazilian individuals and 37 haplotypes from 130 Iranian individuals (Bozorgomid et al. 2020). Eight haplotypes were found within 90 *F. hepatica* samples (based on a 535 bp *nad*1 fragment) and six haplotypes were found within 30 *F. hepatica* samples (based on a 745 *cox*1-*nad*1 concatenated fragment) in previous investigations from Iran and Armenia, respectively (Reaghi et al. 2016; Semyenova et al. 2006). In a Peruvian investigation, the *nad*1 component from 78 distinct parasites was examined. Eight haplotypes were discovered (Hd = 0.685 and π = 0.00175) (Ichikawa-Seki et al. 2016). Seven *cox*1 gene haplotypes were found in an Argentine investigation that included 22 participants. When *nad*4 and *nad*5, two more mitochondrial genes, were examined, four and three haplotypes were found, correspondingly (Carnevale et al. 2017). Only six *cox*1 haplotypes (Hd = 0.482 and π = 0.003) and 18 *nad*1 haplotypes (Hd = 0.832 and π = 0.005) were found in an analysis of 208 samples conducted by Elliott et al. (2014) in Australia. Ichikawa-Seki et al. (2017) discovered that in 211 lamellipodia from different geographic regions of China, 11 haplotypes belonged to an *F. hepatica* population and 18 haplotypes belonged to an *F. gigantica* population.

The existence of *F. hepatica* in Peru, Argentina, Australia, and Southern Brazil with a very limited number of individuals, each from a much larger parental population, producing a Founder's effect, may be one explanation for both high haplotype diversity and low nucleotide diversity. We propose that *F. hepatica* was introduced into Brazil in a number of distinct waves of human and cattle immigration in order to more adequately explain the large number of haplotypes that were observed.

In the present study, the genetic variation and population dynamics of *F. hepatica* were evaluated. This was accomplished by utilizing sequenced information from the *cox*1 and *nad*1 gene sequences that were obtained from GenBank and are frequently utilized for *Fasciola* species differentiation. As a result of this work, knowledge about global gene flow and population dynamics in *F. hepatica* infections was gained. For this in silico analysis, we used a total of 247 *cox*1 (387 bp) and 357 *nad*1 (689 bp) gene sequences of *F. hepatica* isolates that were already registered in the NCBI database to assess the genetic diversity and variation of the *F. hepatica*. The results of the current study show an extremely high global haplotype diversity within both *cox*1 and *nad*1 genes. The 604 samples analyzed represented a total of 46 haplotypes for the *cox*1 and 98 for *nad*1 genes.

Nucleotide diversity and population expansion were assessed by using neutrality indices including Tajima *D*, Fu's Fs, and Fu's LD (Ramos-Onsins and Rozas 2002). A positive Tajima *D* value indicates heterozygosity,

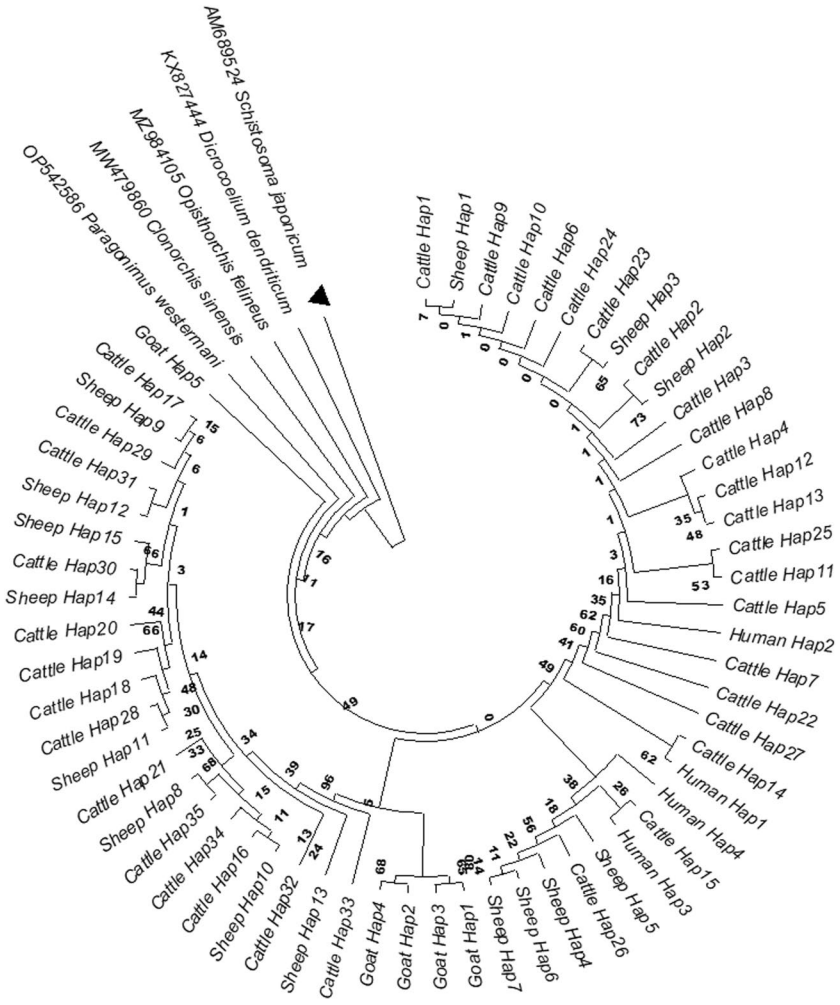

Fig. 5 Evolutionary analysis of host wise investigation of *cox*1 gene sequences inferred by maximum likelihood method

which is described as having a selective advantage, whereas negative values show that one allele has a selective advantage over the other allele. The Tajima *D* test measures how much populations deviate from the traditional neutral model. A negative score also denotes a rapid population increase (Vamathevan et al. 2008; Stephens et al. 2001). Both the *cox*1 and *nad*1 gene segments in our study had high Tajima's *D* values, which suggested a low likelihood of future population growth. The Tajima's *D* value of the *nad*1 gene sequence is lower (2.14910) than that of the *cox*1 gene sequence (3.40314), which suggests that the former is growing at a slower rate. With a significantly negative value ($p < 0.05$), Fu's Fs serves as a marker of population growth sensitivity, showing that the populations share the same gene pool and exhibit similar growth tendencies (Fu 1997; Li et al. 2009). In both the *cox*1 and *nad*1 haplotype groupings, our study produced highly positive and statistically non-significant Fu's Fs values, indicating that these populations have not spread internationally.

The level of polymorphism in the population was found by evaluating nucleotide diversity. We found that the mean nucleotide difference between the *nad*1 (0.21029) and *cox*1 (0.17426) gene sequences was larger. In order to determine the uniqueness of haplotypes within the population, haplotype diversity was also evaluated. The values of the *nad*1 (0.860) and *cox*1 (0.869) gene sequences were extremely comparable in our investigation.

Our investigation of the *cox*1 gene sequences resulted in the discovery of 46 haplotypes in total. The network consisted of 30 individual haplotypes, with the dominant haplotype accounting for 25.91% of the whole. In our examination of the *nad*1 gene sequence, 98 distinct haplotypes were found. There were 76 individual haplotypes, and the predominant haplotype made up 31.65% of the entire network. The major haplotypes all share a common ancestry.

The 387 bp *cox*1 gene sequences had 154 unique mutations found throughout them, whereas the 689 bp *nad*1

Fig. 6 Evolutionary analysis of host wise investigation of *nad*1 gene sequences inferred by maximum likelihood method

sequences had 275 unique mutations found. The longer and more complicated evolutionary history of *F. hepatica* may be reflected in the higher mutation rates. Worldwide, there is a very high level of genetic variation within the *F. hepatica* species, and the complicated phylogeographic patterns that have been identified by phylogenetic and geographic analyses imply that the intensive animal trade has influenced the species' current distribution. Several South American, North African, Asian, and Middle Eastern countries (Ecuador, Brazil, Algeria, Iran, Afghanistan, etc.) had a high number of haplotypes detected in this study, suggesting that *F. hepatica* has been present in these regions for a longer period of time than in some western nations (Italy and Spain).

Region-wise analysis revealed that both the *cox*1 and *nad*1 genes showed deviation from neutrality suggesting a recent population expansion as a result of an excess of low-frequency polymorphism. Furthermore, overall host-wise analysis showed positive and significant Tajima's *D* values for both the *cox*1 and *nad*1 gene sequences. To the best of our knowledge, this is the first attempt to provide insights into genetic variations and population structure of *F. hepatica* at a global scale using *cox*1 and *nad*1 genes. Our findings suggest the existence of specific variants of *F. hepatica* in different parts of the world and provide information on the molecular ecology of *F. hepatica*. In this analysis, 387 bp *cox*1 and 689 bp *nad*1 gene consensus sequences were investigated to describe genetic diversity. In the future, we recommend the utilization of longer, rather complete, gene sequences to provide a broader picture of genetic variations among *F. hepatica* isolates. It is further suggested to study additional mitochondrial markers as the discriminatory power of a set of mitochondrial gene targets is superior to describing genetic diversity.

Conclusion

Communities with a high prevalence of intermediate hosts face a serious threat from *F. hepatica*. This study is the first bioinformatics investigation to assess the genetic structure

F. hepatica gathered globally, despite the fact that numerous molecular studies have been carried out to date. All of the sequencing information from *F. hepatica* isolates relevant to cattle and humans was presented in this study. We assume that this research can close any knowledge gaps in the area. Our findings also mark a critical development in upcoming epidemiological and bioecological research that may result in effective therapies for specific species or strains.

Data availability statement All data supporting the conclusions of this article are included in the article.

Author contributions MAA, AK, LL, and HBY conceptualized the study. The methodology was designed by MAA, MS, RMAA, and BA. Formal analysis was carried out by AK, MAA, RMAA, WQ, and MS. Writing of the original draft was done by MAA and AK while WZJ, HBY, and BQF edited the draft. WZJ supervised the project.

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Declarations

Conflict of interest The authors declare no competing interests.

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