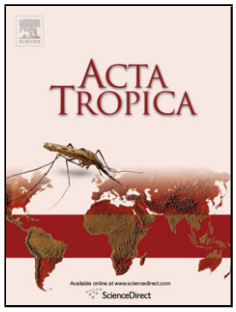


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# Two haplotype clusters of *Echinococcus granulosus* sensu stricto in northern Iraq (Iraqi Kurdistan) support the hypothesis of a parasite cradle in the Middle East.

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**Highlights**

- We confirmed *E. granulosus* s.s. as the cause of hydatidosis in livestock animals from Iraqi Kurdistan.
- The parsimony network displayed a double-clustered topology which was also found to occur in the Middle East, Europe, Mongolia, Russia and Tunisia.
- We hypothesize that this double clustering may have emerged independently and dispersed from the Middle East into Europe, Mongolia, Russia and North Africa.

## ABSTRACT

Human cystic echinococcosis (CE) caused by *Echinococcus granulosus* s.s. is a major public health problem in Iraqi Kurdistan with a reported surgical incidence of 6.3 per 100,000 Arbil inhabitants. A total of 125 *Echinococcus* isolates retrieved from sheep, goats and cattle were used in this study. Our aim was to determine species/genotypes infecting livestock in Iraqi Kurdistan and examine intraspecific variation and population structure of *Echinococcus granulosus* s.s. in this region and relate it to that of other regions worldwide. Using nucleotide sequences of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox 1*) we identified *E. granulosus* s.s. as the cause of hydatidosis in examined animals. The haplotype network displayed a double-clustered topology with two main *E. granulosus* s.s. haplotypes, (KU05) and (KU33). The 'founder' haplotype (KU05) confirmed the presence of a common lineage of non-genetically differentiated populations as inferred by the low non-significant fixation index values. Overall diversity and neutrality indices indicated demographic expansion. We used *E. granulosus* s.s. nucleotide sequences from GenBank to draw haplotype networks for the Middle East (Iran, Jordan and Turkey), Europe (Albania, Greece, Italy, Romania and Spain), China, Mongolia, Russia, South America (Argentina, Brazil, Chile and Mexico) and Tunisia. Networks with two haplotype clusters like that reported here for Iraqi Kurdistan were seen for the Middle East, Europe, Mongolia, Russia and Tunisia using both 827bp and 1609bp *cox1* nucleotide sequences, whereas a star-like network was observed for China and

South America. We hypothesize that the double clustering seen at what is generally assumed to be the cradle of domestication may have emerged independently and dispersed from the Middle East to other regions and that haplotype (KU33) may be the main haplotype within a second cluster in the Middle East from where it has spread into Europe, Mongolia, Russia and North Africa. Further studies using metacestodes of human origin are required to investigate the biological importance of *E. granulosus* s.s. haplotypes/clusters and their association, if any with clinical manifestations of CE infection.

**Keywords:** *Echinococcus granulosus* s.s.; Iraqi Kurdistan; genetic variation

## 1. Introduction

Cystic echinococcosis (CE) caused by the metacestode stage of *Echinococcus granulosus* sensu lato s.l. is a parasitic zoonosis of major importance and worldwide distribution (Craig et al., 2007). Members of *E. granulosus* s.l. species complex include *Echinococcus granulosus* sensu stricto s.s. (genotypes G1-G3), *Echinococcus felidis* ("lion strain"), *Echinococcus equinus* (G4), *Echinococcus ortleppi* (G5) and *Echinococcus canadensis* (G6/G7, G8, G10) (Nakao et al., 2007; Thompson, 2008; Hüttner et al., 2008; Romig et al., 2015). The life cycle of *E. granulosus* s.s. is perpetuated between dogs and ungulates (mainly sheep) that serve as definitive and intermediate hosts, respectively. Infection occurs through the ingestion of the tapeworm eggs that develop into fluid-filled metacestode larvae primarily in the liver and lungs of livestock animals and humans.

Human CE is highly endemic in large parts of Iraq where it has been reported from Bagdad (Senekji and Beattie 1940; Al-Jeboori, 1976; Khalili et al., 1989; Al-Naimi et al., 2012), Basrah (Al-Mukhtar, 1989; Maktoof and Tabeekh, 2015; Thamir et al., 2015), Mosul (Al-Sakkal, 1982; Salih et al., 1983; Younis et al., 2008), Babylon (Molan and Baban 1989), the southern region (Benyan and Mahdi, 1987, Molan, 1993) and Arbil, the capital city of Iraqi Kurdistan in northern Iraq (Al-Barwari et al., 1991; Saeed et al., 2000). Using retrospective admission records of two main hospitals in Arbil, CE surgical incidence for the period between 1990 and 1998 was estimated to be 2 per 100,000 inhabitants (Saeed et al., 2000). A recent retrospective study of patients' records from public and private hospitals showed an incidence of 6.3 per 100,000 inhabitants of Arbil population (Saida and

Nouraddin, 2011). However, few reports on the molecular identification of human cystic echinococcosis causative agent are known to exist (Hama et al., 2012; Ahmed et al., 2013; Baraak, 2014).

CE is also prevalent in ungulate intermediate hosts in many Iraqi provinces (Al-Abbassy et al., 1980; Molan and Saeed 1990; Wajdi and Nassir, 1983; Saeed et al., 2000; Saida and Nouraddin, 2011; Abdulla and Mero, 2013; Mero et al., 2014; Hassan et al., 2016). Additionally, consistently high *E. granulosus* infection rates in stray dogs have been reported over time from various regions of Iraq. In a study carried out in Bagdad, 17.83% of 123 dogs were found to be infected with *E. granulosus* (Senekji and Beattie et al., 1940). Infection rates in dogs from 11 localities in Arbil ranged from 66.7-100% (Molan and Saida, 1989) and an *E. granulosus* prevalence of 56% in 50 stray dogs was reported from Theqar southern province (Molan 1993). The necropsy of 120 stray dogs in Mosul between 1997 and 1999 revealed an infection rate of 16.7% (Abdullah and Jarjees, 2005). To date, molecular epidemiological studies using hydatid isolates from ungulate intermediate hosts from Iraqi Kurdistan have been limited to one report from Slemani Province (Hama et al., 2013). In addition, no molecular confirmatory reports using DNA extracted from adult tapeworms removed from infected dogs at necropsy have been published.

Considerable intraspecific variation is known to exist within *E. granulosus* s.l. (Thompson and McManus, 2002) and the genetic polymorphism of *E. granulosus* s.s. metacestodes has been extensively studied (Nakao et al., 2010; Yanagida et al., 2012; Casulli et al., 2012; Boufana et al., 2014; 2015a; Romig et al., 2015; Mahami-Oskouei et al., 2016; Kinkar et al., 2016; Laurimäe et al., 2016) and to a lesser extent that of the adult tapeworms (Boufana et al., 2015b). This study was conducted to molecularly investigate *Echinococcus* species/genotypes infecting livestock animals in Iraqi Kurdistan and determine the genetic

variation and population structure of *E. granulosus* s.s. in this region and relate the results to those described worldwide.

## 2. Materials and methods

### *2.1. Samples, DNA extraction, PCR amplification and sequencing*

A total of 125 hydatid cyst isolates collected between July 2013 and June 2014 from livestock animals (sheep n = 66; goats n = 24; cattle n = 21) slaughtered in district abattoirs of Arbil and Duhok Provinces in Iraqi Kurdistan and Mosul in the north of Iraq (sheep n = 13; cattle n = 1) were included in this study. Hydatid cysts removed from the liver, lungs and spleen were transported to the laboratory and protoscoleces and/or germinal layers were aseptically collected and stored in 70% ethanol. Total genomic DNA was extracted from hydatid cyst material using the Qiagen DNeasy Blood and Tissue DNA extraction Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A fragment within the mitochondrial cytochrome *c* oxidase subunit 1 (*cox 1*) gene was amplified using published methodology (Nakao et al., 2000). PCR products were electrophoresed in 1.5% (w/v) ethidium bromide stained agarose gels in 1X Tris-Borate-EDTA buffer at 110V and viewed using UV illumination (Syngene G:Box gel documentation system, Cambridge Biosciences). Amplified products were purified using QIAquick PCR Purification Kit (Qiagen) and commercially sequenced in both directions using the PCR primers (Macrogen EZ- Sequence, Amsterdam, The Netherlands). Chromatograms were viewed using FinchTV trace viewer (Geospiza, Seattle, WA, USA) to verify peak quality. The identity of the sequenced nucleotide fragments was ascertained through the use of BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST/).

### *2.2. Combined Echinococcus granulosus s.s. dataset*

To compare *E. granulosus* s.s. isolates from Iraqi Kurdistan with those from other world regions, a broader nucleotide sequence dataset was created to include *E. granulosus* s.s. sequences from the NCBI database (http://www.ncbi.nlm.nih.gov). C*ox 1* mitochondrial nucleotide sequences of *E. granulosus* s.s. metacestodes from Albania n = 2, Greece n = 1, Italy n = 6, Romania n = 1, Spain n = 10, Turkey n = 60 (Kinkar et al., 2016); China n = 62, Iran n = 15, Jordan n = 12 (Yanagida et al, 2012; Wang et al., 2014, unpublished; Mohammadzadeh et al., 2011, unpublished); Mongolia n = 29 (Ito et al., 2014, Narankhajid et al., 2013, unpublished); Russia, Altai n = 7, Novosibirsk Oblast n = 1, Permskiy Krai = 2, Republic of Bashkiria n = 1 (Konyaev et al., 2012; Konyaev et al., 2013); Argentina n = 17, Brazil n = 6, Chile n = 4, Mexico n = 1 (Laurimäe et al., 2016); Chile n = 21 (Alvarez et al., 2017) were used (Table 1). In addition, Tunisian *E. granulosus* s.s. metacestode *cox 1* nucleotide sequences of animal and human origin (n = 123) used in a previous study (Boufana et al., 2014) were also included. The GenBank sequences were of different lengths (1609bp or 1674bp) to those analysed in this study and were, therefore, trimmed to equal lengths and we used 381 *E. granulosus* s.s. *cox 1* nucleotide sequences to generate regional haplotype networks for the Middle East, Europe, China, Mongolia, Russia, South America and Tunisia.

*2.3. Data analysis*

Analysis of sequenced data was carried out using published methodology (Boufana et al., 2014). In brief, data was aligned in ClustalX (Larkin et al., 2007) and transported into DnaSP 5 (Librado and Rozas, 2009). Modeltest 3.7 (Posada and Crandall, 1998) was used to determine the model of evolution executed in Paup 4.0 (Swofford, 1998). Arlequin version 3.5. (Excoffier and Lischer, 2010) was used to determine haplotype numbers (*h*n), and haplotype (*h*d) and nucleotide diversities (πd). Overall genetic differentiation among populations was quantified in Arlequin using the pairwise fixation index (Fst). Demographic

events were evaluated using 3 selective neutrality tests. Population expansion model and bottleneck were tested using Fu's Fs (Fu, 1997) and Tajima's *D* (Tajima, 1989), respectively, in Arlequin using 1000 permutations to test for their significance. The former statistic is based on allele/haplotype distribution and is sensitive to recent population growth whereas the latter incorporates the frequency distribution of segregating nucleotide sites. DnaSP was used to determine Ramos-Onsin's Rozas's $R_2$ (Ramos-Onsins and Rozas, 2002) and the *P*-value with 1000 replicates was determined using coalescence simulations. Hapview (Salzburger et al., 2011) was used to generate haplotype networks to determine relationships between individual haplotypes. DNAML program (PHYLIP) (Felsenstein, 1989), which was run from Hapview, was used to construct maximum likelihood trees.

## 3. Results

### *3.1. Analysis of sequenced data*

We analysed an 827bp *cox1* fragment using BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST/) by comparing our generated nucleotide sequences with those present in the NCBI database (http://www.ncbi.nlm.nih.gov) and confirmed that the DNA amplified from 125 hydatid cyst isolates retrieved from livestock hosts included in this study (sheep, goats, cattle) all belonged to *E. granulosus* s.s. A total of 16 (41%) parsimony informative polymorphic sites were detected within the analysed nucleotide sequences and no indels or gaps were observed.

### *3.2. Iraqi Kurdistan E. granulosus s.s. isolates: haplotype diversities and parsimony network*

A total of 39 polymorphic sites within the aligned 125 *cox 1* nucleotide sequences of *E. granulosus* s.s. were detected identifying 38 haplotypes. Overall, high haplotype and low nucleotide diversity were observed (Table 2). Haplotype diversity was high for all host-

derived cysts with the highest and lowest being reported in goats (0.9058) and cattle (0.7619) isolates, respectively. In contrast, nucleotide diversity was low ranging from 0.0017 in cattle to 0.026 in goat hydatid derived isolates.

The parsimony haplotype network consisted of two centrally-positioned haplotype clusters having KU05 and KU33 as the dominant haplotypes, each with 29/125 (23.2%) and 32/125 (25.6%) hydatid isolates, respectively (Fig. 1). A BLAST search showed haplotype KU05 to have a 100% identity with the common or 'founder' *E. granulosus* Eg01 haplotype reported to be globally distributed (Iran, JQ250806, Yanagida et al., 2012; EgTu01, Tunisia, KM014606, Boufana et al., 2014). Haplotype KU33 had a 100% identity with *E. granulosus* haplotypes from Iran (Eg04, JQ250809, Yanagida et al., 2012), Romania (ROM1, KU925431, Kinkar et al., 2016), Russia, Altai (EgRUS4, AB688139, Konyaev et al., 2012), Mongolia (H01, AB787538, Narankhajid et al., 2013) and Tunisia (EgTu37, KM014642, Boufana et al., 2014). Of the remaining haplotypes, KU04, KU08, KU10, KU12, KU14, KU16-KU17, KU20, KU23-KU24, KU26 and KU36 had a 100% identity with *E. granulosus* (AB688595, AB688597, KM014612, AB688593, AB688607, JQ219963, JQ250815, KM014640, KX020356, KX020376, KU925398, JQ250812) from Jordan, Tunisia, China, Iran, Armenia and Turkey. A further 24 haplotypes, KU01-KU03, KU06-KU07, KU09, KU11, KU13, KU15, KU18-KU19, KU21, KU22, KU25, KU27-KU32, KU34-KU35, KU37, KU38) displayed a 99% identity with *E. granulosus* from Tunisia, Iran, China, Turkey, Russia, Mongolia, Armenia, Chile, Jordan, Argentina and Romania (KM014642, KM014607, KM014627, JQ250806, AB688616, KU925395, AB777908, AB893250, AB893248, JQ250810, KM014639, KX020376, KX227127, KM014623, JQ250814, AB688599, AB688139, JQ219962, JQ250807, KX039951, JQ250809, KR337820, KP751431, KU925431). There were 6 shared haplotypes (KU05, KU11, KU13, KU17, KU27, KU33), 23 unique to sheep (KU01-KU04, KU07, KU09-KU10, KU12, KU14, KU16, KU18, KU20,

KU22-KU26, KU28-KU32, KU38), 7 to goats (KU06, KU15, KU21, KU34-KU37), and 2 to cattle (KU08, KU19). Nucleotide sequences of haplotypes generated in this study (KU01-KU38) were deposited in GenBank (Accession numbers XXXXX-XXXXX).

*3.3. Iraqi Kurdistan E. granulosus* s.s. *isolates:demographic analysis*

Overall significantly negative neutrality indices (Tajima's *D* -2.2329, $p = 0.0006$ and Fu's Fs –27.5031, $p = 0.0000$) were recorded for the 125 *cox1 E. granulosus* s.s. nucleotide sequences which suggested the presence of rare nucleotide variants than would be expected under neutrality, indicating population expansion. In addition, Ramos-Onsins and Rozas's $R_2$, which predicts severe recent population expansion, was small and statistically significant ($R_2$ 0.0239, $p = 0.001$). When indices for *E. granulosus* s.s. isolates from individual hosts were analysed independently, significantly negative Tajima's *D* and Fu's Fs were recorded for *E. granulosus* s.s. isolates from sheep and goat origin supporting population growth (Table 2). Negative but non-significant Tajima's *D* and Fu's Fs were detected for *E. granulosus* s.s. from cattle isolates, which suggested the presence of excess rare mutations, a feature of recent population expansion.

The pairwise comparisons between *E. granulosus* s.s. isolates from sheep, goats and cattle as expressed by the Fst index, which is based on haplotype distribution, indicated that these populations were not genetically differentiated (sheep/goat Fst = 0.0355, $p = 0.0187$; sheep/cattle Fst = 0.0005, $p = 0.3717$; goat/cattle Fst = 0.0083, $p = 0.24602$).

*3.4. Regional haplotype networks*

Regional haplotype networks were drawn in order to investigate the occurrence of the second main *E. granulosus* s.s. haplotype reported in this study (KU33). A nucleotide sequence of a Kurdish isolate occupying haplotype KU33 was included and served as a reference sequence. Seven individual networks were depicted for *E. granulosus* s.s. from the

Middle East (Iran, Jordan and Turkey), Europe (Albania, Greece, Italy, Romania and Spain), China, Mongolia, Russia, South America (Argentina, Brazil, Chile and Mexico) and Tunisia (Fig 2).

Networks like that reported here for Kurdistan with 2 main haplotype clusters were seen for the Middle East, Europe, Mongolia, Russia and Tunisia (Figs 2A-2B, 2D-2E, 2G), whereas the remaining regional networks (China and South America) displayed a star-like network topology with a single main haplotype (Figs 2C and 2F). We also investigated whether the generation of a double-clustered network may have been affected by the use of a relatively short nucleotide sequence (827bp). Based on available database sequences (Table 1), we constructed *cox 1* networks for the Middle East (Iran, Jordan, Turkey), Europe (Albania, Greece, Italy, Romania and Spain), China, Mongolia, Russia and South America (Argentina, Brazil, Chile and Mexico) using 1609bp. Similar *E. granulosus* s.s. double-clustered network topologies were seen for the Middle East, Europe, Mongolia and Russia (data not shown). Tunisian *cox1* nucleotide sequences of similar length (1609) were not available for comparison.

**4. Discussion**

The current study examined the genetic diversity, population structure and demographic history of *E. granulosus* s.s. from Iraqi Kurdistan. Using sequenced data of the *cox 1* mitochondrial gene, we confirmed the presence of *E. granulosus* s.s. in sheep, goats and cattle from Arbil, Duhok and Mosul. Results in this study demonstrate the importance and dominance of *E. granulosus* s.s. within Iraqi Kurdistan and emphasize its prevalence as the causative agent of cystic echinococcosis in animals. Additionally, this study has shown ungulate hosts to possess both unique and genetically identical haplotypes, which is indicative of the rapid and free circulation of *E. granulosus* s.s. among these intermediate hosts in Iraqi Kurdistan and of their importance in the perpetuation of the sheep-dog life

cycle within this region. In addition, to date molecular studies using mitochondrial c*ox 1* and NADH dehydrogenase 1 (*nad1*) sequences generated using DNA extracted from metacestodes removed from CE patients in Kurdistan and other Iraqi provinces have reported *E. granulosus* s.s. as the sole causative agent of cystic echinococcosis in humans (Hama et al., 2012; Ahmed et al., 2013; Baraak, 2014).

The occurrence of a 'founder' *E. granulosus* s.s. haplotype (KU05) described from other geographical regions, was confirmed in this study illustrating the presence of a common lineage of non-genetically differentiated populations supported by inference of gene flow as indicated by the low non-significant Fst values. The high haplotype, low nucleotide diversities observed in this study along with the significantly negative neutrality indices, reflect populations under expansion and has been reported for *E. granulosus* s.s. from various parts of the world (Nakao et al., 2010; Yanagida et al., 2012; Casulli et al., 2012; Boufana et al., 2014; Kinkar et al., 2016).

The presence of *E. granulosus* s.s. in Iraqi Kurdistan as represented by the two main haplotype clusters is not surprising. The Ancient Near East (described by archaeologists as southwest Asia) has been suggested as the 'ancestral seat' of *E. granulosus* s.s. (Eckert et al., 2001; Nakao et al., 2010). It has also been speculated that 'founder' individuals of *E. granulosus* s.s. from the Middle East were dispersed to other regions through the anthropogenic movement of animals (Nakao et al., 2010; Yanagida et al., 2012; Casulli et al., 2012) and therefore it is generally considered that *E. granulosus* s.s. populations from the Middle East display greater genetic diversity due to the longer evolutionary time (Casulli et al., 2012).

However, haplotype diversity seen in this study (n = 125; 827bp; Hd 0.8729) was similar to that computed using variable lengths of mitochondrial nucleotide sequences for *E. granulosus* s.s. isolates from different geographical regions (Table 3). Although variable

sample sizes were used in those studies yet, this geographically similar haplotype diversity does not appear to fit with the founder introduction from the Middle East. The apparent absence of a reduction in genetic diversity further away from the centre of domestication, generally regarded to be the Middle East (Nakao et al., 2010; Yanagida et al., 2012; Casulli et al. 2012) has also been observed for *E. granulosus* s.s. isolates from Europe and is considered to be due to the intensive and rapid animal trade (Kinkar et al., 2016).

The results of the construction of regional networks using *cox1* nucleotide sequences for *E. granulosus* s.s. isolates originating from the Middle East, Europe, Mongolia, Russia and Tunisia, revealed a similar network structure to that described here for the Iraqi Kurdish isolates in relation to the presence of 2 distinct clusters. The *E. granulosus* s.s. regional haplotype networks clearly suggest the presence of a double-clustering for the Middle East. This is also evident in the 2 opposite directions: in the east towards Mongolia/Russia and the west towards Europe (Albania, Greece, Italy, Romania and Spain) but, due to the small sample size for these latter regions, we cannot exclude a more complex haplotype structure like that of the Middle East. A similar network topology seems to be apparent for *E. granulosus* s.s. isolates from Tunisia, although a somewhat more complex clustering, than that indicated for the Middle East, is evident. Further, even when longer mitochondrial sequences (8274bp) were used to examine genetic variation within *E. granulosus* s.s. from Europe, one of the main findings in addition to the high genetic variation, was the general topology of the network, which was composed of 2 haplotype clusters that were still evident even when the authors used shorter sequences (351bp and 1674bp) for comparison purposes (Kinkar et al., 2016). It is also noteworthy, that some of the Turkish *E. granulosus* s.s. isolates (Kinkar et al., 2016) utilized in this study originated from Erzurum and Elazig provinces that border with Iraqi Kurdistan. This geographical proximity may also explain the similarity in the network topology seen here for the Middle East. In contrast, haplotype

networks for China and Argentina were dominated by a single cluster within a star-like topology, which is in agreement with previous reports from these regions (Nakao et al., 2010; Yanagida et al., 2012; Laurimäe et al., 2016).

We hypothesize that the double clustering of haplotypes seen at what is generally assumed to be the cradle of domestication may have emerged independently and dispersed from the Middle East to other regions. Further, these two clusters may have been in existence at the time when *E. granulosus* s.s. undertook a host change from wild to domesticated animal species or when a host switch may have occurred between domesticated animals such as sheep, goats and cattle.

Within the second cluster, haplotype KU33 was the most prevalent haplotype seen in this study in terms of isolate numbers (n = 32). A BLAST search showed this haplotype to be 100% identical to haplotypes described from Iran, Romania, Russia and Mongolia and was seen in the regional networks clustering with genetically similar isolates from these countries (Figs 2A-2B, 2D-2E, 2G). Interestingly, it was also 100% identical to a Tunisian haplotype (EgTu37), which encompassed the highest number of fertile isolates of *E. granulosus* s.s. in donkeys (Boufana et al., 2014). We hypothesize that haplotype KU33 generated here using 827bp *E. granulosus* s.s. *cox1* nucleotide sequences may be the main haplotype within a second cluster in the Middle East from where it spread into Europe, Mongolia, Russia and North Africa. Archaeological data suggests that sheep were domesticated almost 9,000 years ago, in the Near East (Smith, 1998) from where they are thought to have dispersed into Europe, Africa and Asia (Chessa et al., 2009) and northern Europe across Russia (Tapio et al., 2006). Further to this, the Mongolian Plateau is thought to have functioned as a 'secondary centre for dispersal' facilitating sheep migration through the Caucasus and Central Asia into China (Lv et al., 2015).

Most of the reports on genetic variation of the metacestode stage of *E. granulosus* s.s. published in recent years and previously cited here, were based on the analysis of variable fragment sizes of a polymorphic mitochondrial gene sequence (*cox 1*). The most relevant findings of these reports were the existence of a common population genetic scenario for *E. granulosus* s.s. described from various parts of the world. This relates to the presence of a 'founder' haplotype of *E. granulosus* s.s. widely prevalent in the place of domestication and spread across continents by historical anthropogenic movements of hosts; the introduction of founder individuals mainly followed by population expansion and high haplotype diversity mostly reflecting single mutational changes within genetically undifferentiated populations. These general findings have also been recently recorded by authors using longer nucleotide sequences spanning several mitochondrial genes (Kinkar et al., 2016; Laurimäe et al., 2016).

The biological significance if any of the numerous haplotypes and/or haplotype clusters described for *E. granulosus* s.s. is currently unknown. Further studies using metacestodes of human origin are required to investigate the importance, relationship and potential relevance of *E. granulosus* s.s. haplotypes/clusters and their association, if any with clinical manifestations of CE infections.

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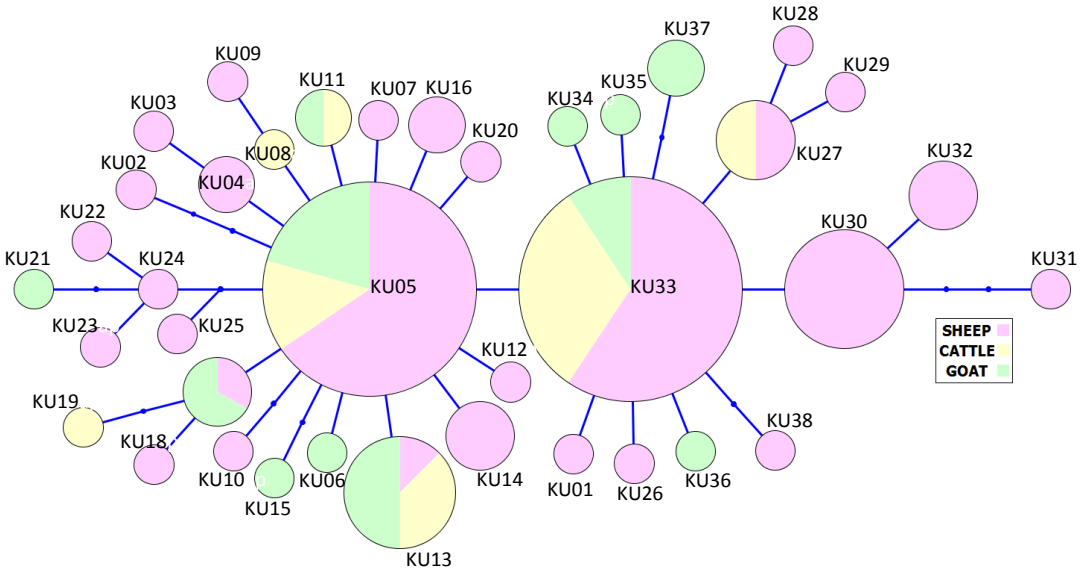


**Fig. 1.** Haplotype network generated using cytochrome *c* oxidase subunit 1 (827bp) mitochondrial nucleotide sequences of *Echinococcus granulosus* sensu stricto from sheep, goat and cattle hydatid isolates from Iraqi Kurdistan.

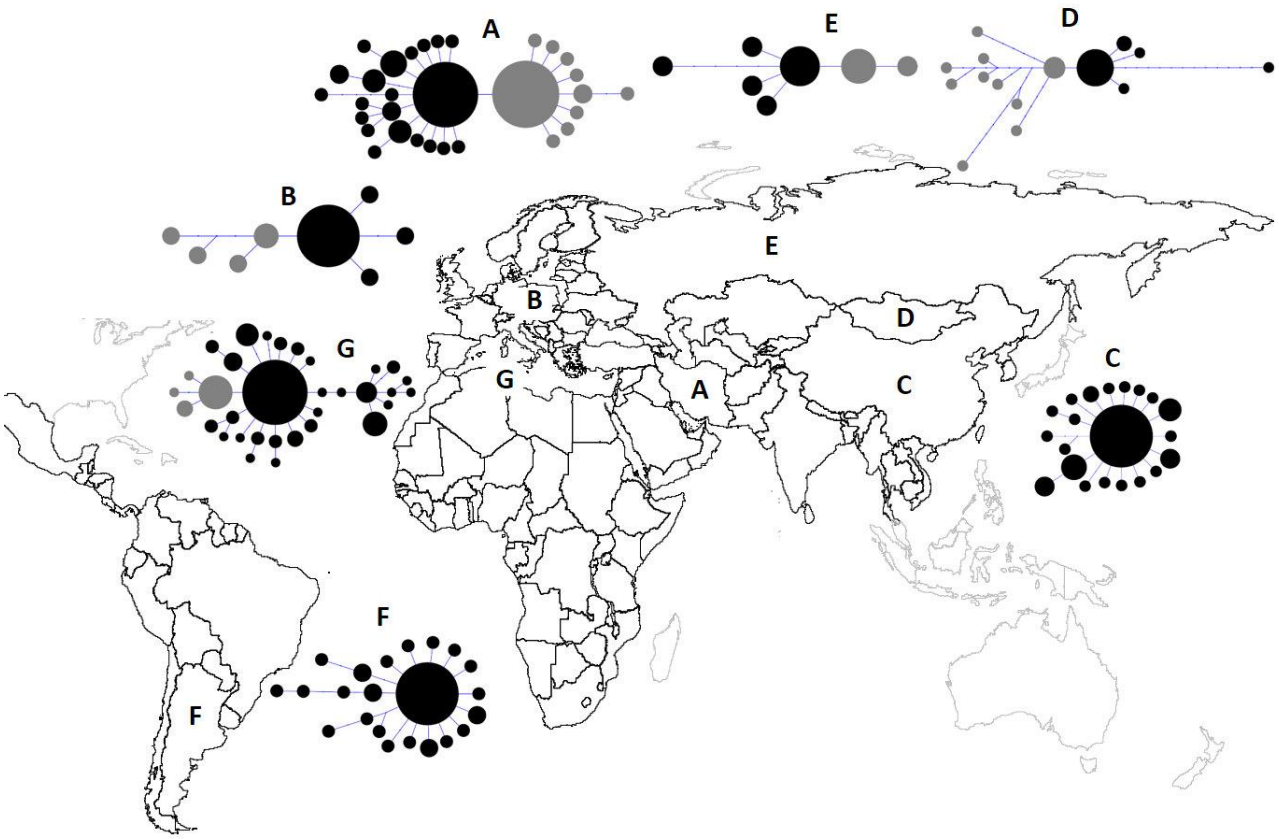


**Fig. 2.** Regional haplotype networks of *Echinococcus granulosus* sensu stricto metacestode isolates (n = 381) from various geographical areas using cytochrome *c* oxidase subunit 1 (827bp). A) the Middle East: Iran n = 15, Jordan n = 12, Turkey n = 60; B) Europe: Albania n = 2, Greece n = 1, Italy n = 6, Romania n = 1, Spain n = 10; C) China, n = 62; D) Mongolia, n = 29; E) Russia, n = 11;
F) South America: Argentina n = 17, Brazil n = 6, Chile n = 25, Mexico n = 1; G) Tunisia, n = 123.
In black: the 'founder' cluster with derived haplotypes. In grey: the second haplotype cluster with derived haplotypes.

**Table 1**
*Echinococcus granulosus* sensu stricto nucleotide sequence data retrieved from GenBank database
and used in this study.

| Countries | GenBank Accession numbers | Reference |
|---|---|---|
| Albania | KU925432-KU925433 | Kinkar et al. 2016 |
| Argentina | KX039937-KX039953 | Laurimäe et al., 2016 |
| Brazil | KX039955-KX039960 | Laurimäe et al., 2016 |

| | | |
|---|---|---|
| Chile | KX039961-KX039964 | Laurimäe et al., 2016 |
| Chile | KX227116-KX227136 | Alvarez et al., 2017 |
| China | AB688602-AB688619 | Yanagida et al. 2012 |
| | KJ628328- KJ628335, KJ628337-KJ628357, KJ628359-KJ628373 | Wang et al., 2014, unpublished |
| Greece | KU925430 | Kinkar et al. 2016 |
| Iran | JQ250806-JQ250817 | Yanagida et al. 2012 |
| | JQ219962-JQ219964 | Mohammadzadeh et al., 2011, unpublished |
| Italy | KU925423-KU925428 | Kinkar et al. 2016 |
| Jordan | AB688590-AB688601 | Yanagida et al. 2012 |
| Mexico | KX039965 | Laurimäe et al., 2016 |
| Mongolia | AB893242-AB893251 | Ito et al., 2014 |
| | AB787529-AB787536 | Narankhajid et al., 2013, unpublished |
| | AB787538-AB787548 | |
| Romania | KU925431 | Kinkar et al. 2016 |
| Russia | AB688136-AB688141 | Konyaev et al., 2012 |
| | AB777904-AB777908 | Konyaev et al., 2013 |
| Spain | KU925413-KU925422 | Kinkar et al. 2016 |
| Turkey | KU925351-KU925360, KU925362-KU925368, KU925370-KU925387, KU925389- KU925398, KU925400- KU925412, KU925361 KU925369 | Kinkar et al. 2016 |

**Table 2**
Diversity and neutrality indices for *Echinococcus granulosus* sensu stricto populations from Iraqi Kurdistan using nucleotide data of the cytochrome *c* oxidase subunit 1 mitochondrial gene.

| Indices | Sheep | Goat | Cattle | Overall |
|---|---|---|---|---|
| No. of isolates | 79 | 24 | 22 | 125 |
| Number of polymorphic sites | 29 | 16 | 8 | 39 |
| No of haplotypes | 28 | 12 | 7 | 38 |
| Haplotype diversity ±S.D | 0.8744±0.0241 | 0.9058±0.038 | 0.7619±0.0768 | 0.8729±0.0199 |
| Nucleotide diversity±S.D | 0.0024±0.0015 | 0.0026±0.0017 | 0.0017±0.0012 | 0.00234±0.0015 |

| | | | | |
|---|---|---|---|---|
| Tajima's *D* | -2.0707 | -1.7752 | -1.1977 | -2.2329 |
| *P*-value | 0.0031[b] | 0.0211[a] | 0.1163 | 0.0006[c] |
| Fu's Fs | -25.7614 | -6.1511 | -2.1595 | -27.5031 |
| *P*-value | 0.0000[c] | 0.0008 [c] | 0.0633 | 0.0000[c] |
| Ramos-Onsins and Rozas's $R_2$ | NC | NC | NC | 0.0239 |
| *P*-value | | | | 0.0010[b] |

[a]*P*<0.05; [b]*P*<0.01; [c]*P*<0.0001; NC, not computed

**Table 3**
Data (isolate numbers, haplotype number, haplotype diversity) using mitochondrial nucleotide sequences for isolates of *Echinococcus granulosus* sensu stricto from various geographical locations.

| Country | Isolate numbers | Haplotype number | Target gene | Amplicon size (bp) | Haplotype diversity | Reference |
|---|---|---|---|---|---|---|
| Bulgaria | 132 | 16 | | 351 | 0.793±0.024 | |
| Hungary | 35 | 11 | *cox 1* | 351 | 0.902±0.037 | Casulli et al., 2012 |
| Romania | 56 | 9 | | 351 | 0.848±0.021 | |
| Serbia | 40 | 7 | *cox 1* | 351 | 0.7526±0.0444 | Debeljak et al., 2016 |
| Iran‡ | 62 | 7 | *cox 1* | 450 | 0.947±0.092 | Mahami-Oskouei et al., 2016 |
| Turkey | 30 | 14 | | 450 | 0.989±0.172 | |
| China | 181 | 43 | *cox 1* | 789 | 0.702 ± 0.038 | Nakao et al., 2010 |
| Tunisia‡ | 145 | 39 | *cox 1* | 827 | 0.8157±0.0315 | From data reported in Boufana et al., 2014 |
| China | 29 | 20 | | 1609 | 0.941±0.034 | |
| Iran | 35 | 12 | *cox 1* | 1609 | 0.887±0.026 | Yanagida et al., 2012 |
| Jordan | 26 | 16 | | 1609 | 0.942±0.027 | |
| Chile‡ | 69 | 26 | *cox 1* | 1609 | 0.875±0.032 | Alvarez et al., 2017 |
| Australia‡ | 37 | 12 | *cox 1* | 1609 | 0.884±0.028 | Alvarez et al., |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | 2016 |
| Europe* | 91 | 83 | mtDNA | 8274 | 0.997±0.002 | Kinkar et al., 2016 |
| Argentina | 36 | 18 | mtDNA | 8279 | 0.805±0.069 | |
| Brazil | 9 | 7 | mtDNA | 8279 | 0.917±0.092 | Laurimäe et al., 2016 |
| Chile | 6 | 4 | mtDNA | 8279 | 0.867±0.129 | |

*includes hydatid isolates from Albania, Greece, Italy, Romania, Spain and Turkey. ‡ Data is for metacestodes and
adult tapeworms. *cox 1*, cytochrome *c* oxidase subunit 1; mtDNA, mitochondrial DNA including *cox 1* gene.