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Canine echinococcosis: genetic diversity of *Echinococcus granulosus* sensu stricto (s.s.) from definitive hosts

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Abstract

Canids, particularly dogs, constitute the major source of cystic echinococcosis (CE) infection to humans, with the majority of cases being caused by *Echinococcus granulosus* (G1 genotype). Canine echinococcosis is an asymptomatic disease caused by adult tapeworms of *E. granulosus* sensu lato (s.l.). Information on the population structure and genetic variation of adult *E. granulosus* is limited. Using sequenced data of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) we examined the genetic diversity and population structure of adult tapeworms of *E. granulosus* (G1 genotype) from canid definitive hosts originating from various geographical regions and compared it to that reported for the larval metacestode stage from sheep and human hosts. *Echinococcus granulosus* (s.s) was identified from adult tapeworm isolates from Kenya, Libya, Tunisia, Australia, China, Kazakhstan, United Kingdom and Peru, including the first known molecular confirmation from Gaza and the Falkland Islands. Haplotype analysis showed a star-shaped network with a centrally positioned common haplotype previously described for the metacestode stage from sheep and humans, and the

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neutrality indices indicated population expansion. Low Fst values suggested that populations of adult *E. granulosus* were not genetically differentiated. Haplotype and nucleotide diversities for *E. granulosus* isolates from sheep and human origin were twice as high as those reported from canid hosts. This may be related to self-fertilization of *E. granulosus* and/or to the longevity of the parasite in the respective intermediate and definitive hosts. Improved nuclear single loci are required to investigate the discrepancies in genetic variation seen in this study.

Introduction

Cystic echinococcosis (CE) is caused by a group of cryptic species within *Echinococcus granulosus* sensu lato (s.l.) including *E. granulosus* sensu stricto (s.s.) (G1–G3 genotypes), *E. equinus* (G4 genotype), *E. ortleppi* (G5 genotype) and *E. canadensis* (G6–G10 genotypes) (Nakao *et al.*, 2007; Thompson, 2008). Human CE due to infection with the metacestode stage of *E. granulosus* (s.l.) is an important zoonotic infection of major health and socio-economic impact worldwide (Budke *et al.*, 2006; Craig *et al.*, 2007). Conversely, canine echinococcosis is caused primarily by the adult stage of *E. granulosus* (s.l.) and the disease is usually asymptomatic in canid definitive hosts, even in those animals harbouring large worm burdens. Although canids and ungulates, respectively, serve as definitive and intermediate hosts of *E. granulosus* (s.l.), dogs and sheep are by far the most important hosts worldwide. Furthermore, infected dogs constitute the major source of CE infection to humans and are known to shed in excess of 8000 eggs per day (Gemmell, 1990). Detection of infection in dogs has traditionally relied on necropsy or arecoline purgation for assessing *Echinococcus* worm burdens (Eckert *et al.*, 2001), but it also relies increasingly on the use of coproELISA for the detection of *Echinococcus* copro-antigens in order to determine prevalence and re-infection rates in dogs (Craig *et al.*, 1995; Allan & Craig 2006; Moss *et al.*, 2013).

Molecular genotypic information on *Echinococcus* isolates from definitive hosts is important for epidemiological and transmission studies, as well as for the planning and surveillance of control programmes (Craig *et al.*, 2003). Published reports on the molecular characterization of *E. granulosus* (s.s.) in definitive hosts include those from Asia (Štefanić *et al.*, 2004; Bart *et al.*, 2006a; Zhang *et al.*, 2006; Ma *et al.*, 2008; Utuk *et al.*, 2008; Ziadinov *et al.*, 2008), the Middle East (Al-Qaoud *et al.*, 2003; Parsa *et al.*, 2012), North Africa (Lahmar *et al.*, 2009; Boufana *et al.*, 2014), Europe (Trachsel *et al.*, 2007; Sherifi *et al.*, 2011; Xhaxhiu *et al.*, 2011) and the Americas (Soriano *et al.*, 2010; de la Rue *et al.*, 2011). In contrast, molecular data on *E. granulosus* (s.s.) from definitive hosts in Sub-Saharan Africa are limited (Wachira *et al.*, 1990, 1993a) compared to data from wild carnivores and domestic and wild herbivores (Wachira *et al.*, 1993b; Hüttner *et al.*, 2009; Kagendo *et al.*, 2014).

The study of the population structure of *E. granulosus* is important as genetic variation is thought to be related to host infectivity (Thompson & McManus, 2002). Using DNA extracted from protoscoleces and/or the germinal layer of *Echinococcus* hydatid cysts retrieved from livestock and human hosts, researchers investigated the genetic diversity of *E. granulosus* (s.s.) from various regions (Nakao *et al.*, 2010; Casulli *et al.*, 2012; Yanagida *et al.*, 2012; Konyaev *et al.*, 2013; Boufana *et al.*, 2014, 2015). These studies highlighted the distribution of *E. granulosus* haplotypes and demonstrated the existence of a single lineage for *E. granulosus* (s.s.) across many geographically isolated regions. In contrast, genetic variation and population structure using *E. granulosus* DNA derived from adult worms and/or canid faecal samples has, to date, not been investigated very widely (Boufana *et al.*, 2014, 2015). Such studies are important in assessing the risk of infection to humans, as the majority of human CE cases are known to be caused by *E. granulosus* (s.s.) (G1 genotype). In a recent study, 88% of 1661 human CE isolates worldwide, were considered to be caused by *E. granulosus* (G1 genotype) (Alvarez Rojas *et al.*, 2014). In the current study we examined the genetic diversity of adult stages of *E. granulosus* (s.s.) from canid definitive hosts originating from various geographical regions, and compared data generated with that reported for *E. granulosus* (s.s.) larval stage metacestodes from sheep and human hosts.

Materials and methods

Sample collection

The origins of the 87 *E. granulosus* isolates used in this study are shown in table 1. Adult tapeworms ($n = 40$) were retrieved at necropsy from stray and semi-stray domestic dogs and jackals (*Canis aureus*) from Tunisia ($n = 22$), dingos (*C. familiaris* var. dingo) from Australia ($n = 11$) and owned dogs from China ($n = 3$) and Kazakhstan ($n = 4$). In addition, a total of 47 coproDNA isolates were used in this study. This faecal panel included domestic dogs from Kenya ($n = 2$), Libya ($n = 9$), China ($n = 1$), Gaza (Palestine) ($n = 3$), the Falkland Islands ($n = 4$), Lima (Peru) ($n = 5$) (Reyes *et al.*, 2012), as well as faeces from Australian dingos ($n = 5$). Faecal samples from Welsh farm dogs ($n = 5$) from a hydatid study carried out in Powys county, Wales (United Kingdom) between May 2008 and July 2010, and from farm sheep dogs ($n = 12$) from the Welsh counties of Powys and Gwent collected between July and November 2002 (Buishi *et al.*, 2005) were also included. A foxhound (dog) faecal sample ($n = 1$) used in this study was collected from an anonymous Welsh hunt in 2011 (Lett, 2013). In addition, DNA extracted from sheep hydatid isolates from Benghazi (Libya) ($n = 13$) and the Falkland Islands ($n = 4$) were available for this study.

Table 1. Number and source of *Echinococcus granulosus* (s.s.) isolates from canid hosts.

	DNA source/No. of isolates		
Country/location (host)	Faeces	Adult worms	Total
Kenya	2	–	2
Libya			9
Benghazi	5	–	
Tripoli	4	–	
Tunisia			22
Ariana, Tozeur, Siliana	–	20	
Jendouba (jackals)	–	2	
Australia			16
Queensland	–	5	
Australian Capital Territory	–	2	
New South Wales	5	4	
China			4
Qinghai	1	3	
Kazakhstan	–	4	4
United Kingdom			18
Wales			
(farm dogs)	17	–	
(foxhound)	1	–	
Palestine			3
Gaza	3	–	
Falkland Islands	4	–	4
Peru			5
Lima	5	–	
Total	47	40	87

Molecular methods

CoproDNA was extracted from faecal samples using the QIAamp DNA stool mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genomic DNA was extracted from ethanol-fixed tissue (adult tapeworms, protoscoleces or membranes of an individual hydatid cyst) using the Qiagen DNeasy Blood and Tissue Kit (Qiagen). DNA was used as template to amplify a fragment within the cytochrome *c* oxidase subunit 1 (*cox1*, 828 bp) mitochondrial gene, as described previously (Nakao *et al.*, 2000). Amplified products were sequenced commercially in both forward and reverse directions (Source Bioscience, Nottingham, UK) and chromatograms were examined using FinchTV viewer (Geospiza, Seattle, Washington, USA).

Data analysis

Data analysis was carried out as described previously (Boufana *et al.*, 2014). In brief, sequence alignments were carried out using MEGA version 6 (Tamura *et al.*, 2013) and ClustalX2 (Larkin *et al.*, 2007) and exported into DnaSP 5 (Librado & Rozas, 2009). Hapview (Salzburger *et al.*, 2011) was used to generate haplotype networks. DNAML program (PHYLIP) (Felsenstein, 1989), which was run from Hapview, was used to construct maximum likelihood trees. Arelquin 3.1 (Excoffier *et al.*, 2005) was used to calculate the number of haplotypes (*h*n), haplotype diversities (*h*d) and nucleotide diversities (πd) as well as to estimate Tajima's *D* (Tajima, 1989) and Fu's Fs (Fu, 1997) in order to examine past population demography and test for neutrality departures. The pairwise fixation index (Fst) was generated in Arlequin to test for genetic differentiation. *P* values for the multiple pairwise comparisons were adjusted using Bonferroni correction.

To compare *E. granulosus* haplotypes from canid hosts with those found in sheep and humans, *cox1* mitochondrial nucleotide sequences publically available on the National Center for Biotechnology Information (NCBI) database (www. ncbi.nlm.nih.gov) were downloaded for inclusion (table 2). Nucleotide sequences were grouped by host (canid, sheep and human) and geographical origin according to six broad regions, Africa, Australia, Asia, Europe, the Middle East and South America. All sequences were trimmed to equal length (329 bp), giving a final dataset of 408 *E. granulosus cox1* mitochondrial sequences, 95 from canids, 180 from sheep and 133 from humans. Rarefied haplotype richness, standardized to the smallest sample size, was calculated using Contrib 1.02 (Petit *et al.*, 1998) in order to facilitate direct comparisons between hosts.

Results

DNA sequence data

We amplified DNA extracted from adult tapeworms and/or faecal samples of 87 *Echinococcus* isolates from canids originating from Africa (Kenya, $n = 2$; Libya, $n = 9$; Tunisia, $n = 22$), Australia ($n = 16$), Asia (China, $n = 4$; Kazakhstan, $n = 4$), United Kingdom ($n = 18$), the Middle East (Gaza, Palestine, $n = 3$) and South America (Falkland Islands, $n = 4$; Peru, $n = 5$). A total of 827 bp within the *cox1* gene were analysed for each *Echinococcus* canid-derived isolate. Using a BLAST search (http:www. blast.ncbi.nlm.nih.gov), *E. granulosus* (s.s.) was identified from adult tapeworms and faecal samples of all canid hosts included in this study. In addition, sheep hydatid cysts from Libya and the Falkland Islands had a 100% sequence identity to *E. granulosus* (G1 genotype) (e.g. accession numbers AB688603, AB893250).

Analysis of E. granulosus *(s.s.) from canid hosts*

Within the 827-bp *cox1* nucleotide sequences analysed there were 18 polymorphic sites, 9 were parsimony informative and 9 were singleton variable sites. The overall haplotype and nucleotide diversities for *E. granulosus* (s.s.) isolates from canid hosts were 0.4405 ± 0.0674 and 0.000958 ± 0.000777, respectively. Similarly, the overall values for Tajima's *D* (-2.26101) and Fu's Fs (-11.8484) were significantly negative (*P* values ≤ 0.0002 and ≤ 0.0001, respectively), a feature indicative of population expansion.

A total of 14 haplotypes (EgCN01–EgCN14) were detected within the 87 canid-derived *E. granulosus* (s.s.) *cox1* mitochondrial sequences (fig. 1a). The frequency of the common haplotype (EgCN04) within the generated star-shaped network was (74.7%; 65/87) and it was 100% identical to the dominant *E. granulosus* haplotypes described from China (G01: AB491414; 789 bp) (Nakao *et al.*, 2010), Iran (Eg01: JQ250806; 1609 bp) (Yanagida *et al.*, 2012), Europe (EG1: JF513058; 351 bp) (Casulli *et al.*, 2012) and Tunisia (EgTu01: KM014606; 827 bp)

Table 2. *Echinococcus granulosus* (s.s.) cytochrome *c* oxidase subunit 1 (*cox1*) mitochondrial nucleotide sequences used in this study.

Region/countries	Canid (*n*)	Sheep (*n*)	Human (*n*)	References
Africa				
Kenya	This study (2)	–	–	–
Libya	This study (9)	This study (13)	HM636641 (1)	Abushhewa *et al.* (2010)
Tunisia	(22)*	(33)*	(24)*	Boufana *et al.* (2014)
Australia	This study (16)	AJ508005–AJ508006, AJ508009–AJ508010 (4)	–	Obwaller *et al.* (2004)
Asia				
China	This study (4), DQ356881, DQ356882 (2)	AB491414–AB491418, AB491421, AB491424–AB491425, AB491432, AB491438, AB491449, AB491454, AB688612 (13)	AB688602–AB688611, AB688613–AB688619, AB491419–AB491420, AB491422–AB491423, AB491428–AB491431, AB491434–AB491437, AB491439–AB491447, AB491451–AB491453, AB491455 (42)	Bart *et al.* (2006a); Nakao *et al.* (2010); Yanagida *et al.* (2012)
Kazakhstan	This study (4)	–	–	–
Europe				
UK	(18)*	(10)*	(4)*	Boufana *et al.* (2015)
Austria	_	JF513058, JF513060, JF513061 (3)	JF513058, JF513060–JF513061, AJ508018–AJ508019, AJ508021–AJ508028 (13)	Casulli *et al.* (2012); Obwaller *et al.* (2004)
Bulgaria	_	JF513058–JF513063, JF513065, JF513071, JF513076, JF513078 (10)	JF513058– JF513063, JF513065 (7)	Casulli *et al.* (2012)
Hungary	_	JF513058–JF513061, JF513063–JF513064, JF513065, JF513067 (8)	JF513058- JF513061, JF513063, JF513065, JF513067, JF690976 (8)	Casulli *et al.* (2012); Snábel *et al.* (2011, unpublished)
Italy	_	JF513058, JF513059–JF513060, JF513062, DQ062857 (5)	JF513058, JF513060, JF513062 (3)	Casulli *et al.* (2012); Varcasia et al. (2005, unpublished)
Portugal	_	JF513058, JF513060, JF513079, HF947594–HF947597, HF947556, HFD947584–HF947585, HF947582, HF947579–HF947580 (13)	JF513058, JF513060, JF513079 (3)	Casulli *et al.* (2012); Beato *et al.* (2013)
Romania	_	JF513058–JF513061, JF513063, JF513072, JF513079 (7)	JF513058–JF513061, JF513063, JF513079, JF520817–JF520818, AY686564–AY686565 (10)	Casulli *et al.* (2012); Bart *et al.* (2006b); Snábel *et al.* (2011, unpublished)
Turkey	_	JF513058, JF513060–JF513062, JF513067, JF513071, JF513079, JF775380, EU929083 (9)	JF513058, JF513060- JF513062, JF513067, JF513079, JF775379, GU951512–GU951513 (9)	Casulli *et al.* (2012); Snábel *et al.* (2009); Arikoglu & Arslan (2008, unpublished); Simsek *et al.* (2011)
Middle East				
Palestine	This study (3)**	KC109640–KC109641, KC109643, KC109645, KC109647-KC109651, KC109653–KC109655,KC109657, KC109659 (14)†	–	Adwan *et al.* (2013)
Iran	JN604097–JN604099, JN604100, JN604101, JN604102 (6)	JQ250806, JQ250808–JQ250809, JQ250811, JQ250813, JQ250816–JQ250817, AB677806, AB677808–AB677810, AB677812 (12)	JQ250810, JQ250812, JQ250815, AB677811, AB677814, KJ540228 (6)	Parsa *et al.* (2012); Yanagida *et al.* (2012); Pezeshki *et al.* (2011, unpublished); Mahami Oskouei *et al.* (2014, unpublished)
Jordan	–	AB688590–AB688601 (12)	–	Yanagida *et al.* (2012)
South America				
Falkland Islands	This study (4)	This study (4)	–	–
Peru	This study (5)	AB458672, AB470527, AB688621, GU233944-GU233950 (10)	AB458675, GU233951– GU233952 (3)	Reyes *et al.* (2012); Moro *et al.* (2009); Yanagida *et al.* (2012); Sanchez *et al.* (2009, unpublished)

n, number of nucleotide sequences.

*The original *cox1* nucleotide sequences (used in the relevant reference to generate *Echinococcus granulosus* haplotypes from Tunisia and United Kingdom) were included in the analysis.

**Gaza; †Nablus.

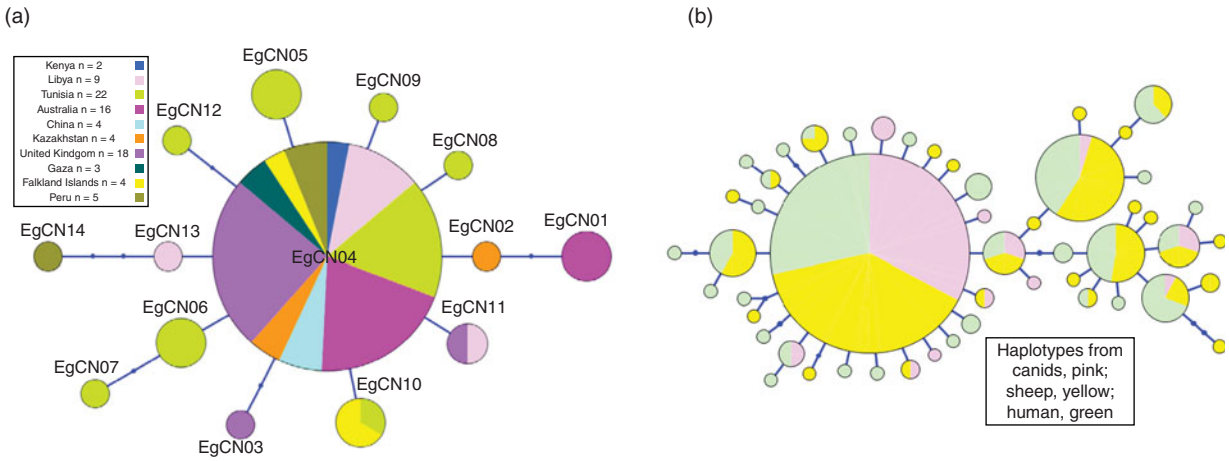

Fig. 1. Haplotype networks generated using cytochrome *c* oxidase subunit 1 (*cox1*) mitochondrial nucleotide sequences of *Echinococcus granulosus* (s.s.) from (a) adult tapeworm isolates derived from canid hosts from various geographic locations; and (b) from adult tapeworms and larval metacestode-stage isolates from sheep and human hosts. Circle size is relative to haplotype frequency. Small circles indicate additional mutational steps.

(Boufana *et al.*, 2014). Haplotypes EgCN01–EgCN14 were deposited in the NCBI database under accession numbers KT001395–KT001408.

The diversity and neutrality indices determined for *E. granulosus* (s.s.) isolates from canid hosts originating from various geographical localities using the *cox1* mitochondrial nucleotide sequences are shown in table 3. The haplotype diversity for the *cox1* gene was highest in samples of adult *E. granulosus* from North Africa (including two Kenyan isolates) and lowest in samples from the United Kingdom. The values for Tajima's *D* were negative for *E. granulosus* samples from all geographical localities (except Australia), indicating population expansion, but remained significant only for samples from Africa and the United Kingdom. Fu's Fs values were also negative for all populations (except Australia and South America), which indicated further evidence of population expansion, but deviated significantly from neutrality only for samples from Africa. Tajima's *D* and Fu's Fs were positive for *E. granulosus* from Australian dingos which suggests that populations may have undergone a genetic bottleneck. Similar positive Fu's Fs values were observed for South American *E. granulosus* canid populations. No conclusion could be drawn for the diversity and neutrality indices of *E. granulosus* isolates from Gaza due to the small sample size of the Palestinian isolates ($n = 3$).

Genetic differentiation based on the analysis of the pairwise Fst values for the *E. granulosus* (s.s.) adult tapeworm isolates from canid hosts originating from different geographical regions are shown in table 4. Generally low, non-significant values were recorded for most pairwise comparisons (following Bonferroni correction), suggesting little genetic differentiation globally.

Comparison of E. granulosus *(s.s.) canid-derived haplotypes to those from sheep and human hosts*

A total of 408 *cox1* nucleotide sequences derived from canid, sheep and human isolates were analysed (tables 2 and 5). The overall haplotype and nucleotide diversities were 0.6631 ± 0.0259 and 0.003618 ± 0.002571, respectively. Within the *cox1* nucleotide sequences there were 45 polymorphic sites, 20 of which were parsimony informative.

Genealogical relationships between *E. granulosus* (s.s.) isolates by host (canid, sheep, human) originating from Africa, Australia, Asia, Europe, the Middle East and South America are shown in fig. 1b. The generated network was

Table 3. Diversity and neutrality indices for *Echinococcus granulosus* (s.s.) adult tapeworm isolates from canid hosts originating from various geographical regions using nucleotide data of the cytochrome *c* oxidase subunit 1 (*cox1*) mitochondrial gene.

Geographical region (*n*)	*h*n	*h*d ± SD	πd ± SD	Tajima's *D*	*P* value	Fu's Fs	*P* value
Africa (Kenya, Libya, Tunisia) (33)	10	0.6288 ± 0.0944	0.001134 ± 0.000889	−2.06508	0.0037**	−7.00817	0.0000***
Australia (16)	2	0.3250 ± 0.1251	0.001183 ± 0.000947	0.22735	0.6525	2.64424	0.8832
Asia (China, Kazakhstan) (8)	2	0.2500 ± 0.1802	0.000303 ± 0.000429	−1.05482	0.2126	−0.18197	0.1989
United Kingdom (18)	3	0.2157 ± 0.1241	0.000404 ± 0.000472	−1.71304	0.0203*	−1.02496	0.0564
South America (Falkland Islands, Peru) (9)	3	0.5556 ± 0.1653	0.001550 ± 0.001213	−1.29379	0.1183	0.8240	0.6584

n, number of isolates, *h*n, number of haplotypes; *h*d, haplotype diversity; πd, nucleotide diversity; SD, standard deviation.
Significant at *P value ≤ 0.05, **P value ≤ 0.01, ***P value ≤ 0.0001.

Table 4. Pairwise fixation index (Fst) for *Echinococcus granulosus* (s.s.) adult tapeworm isolates from canid hosts originating from various geographical regions using nucleotide data of the cytochrome *c* oxidase subunit 1 (*cox1*) mitochondrial gene.

Geographical regions	Africa	Australia	Asia	United Kingdom	South America
Africa (Kenya, Libya, Tunisia)	–				
Australia	0.0943				
Asia (China, Kazakhstan)	−0.0207	0.0058			
United Kingdom	0.0078	0.1095	−0.0095		
South America (Falkland Islands, Peru)	0.0314	0.0940	0.0242	0.0795	–

Non-significant at corrected P value ≤ 0.003.

made up of 51 haplotypes, with the most common central haplotype encompassing 56.6% (231/408) of the total number of *E. granulosus* isolates. The percentage of canid, sheep and human isolates within the dominant haplotype were 32.9%, 41.6% and 25.5%, respectively. A BLAST search showed this dominant haplotype to be 100% identical to that of the common *E. granulosus* haplotype described by other researchers (Nakao *et al.*, 2010; Casulli *et al.*, 2012; Yanagida *et al.*, 2012; Boufana *et al.*, 2014). The network was also made up of a smaller number of more highly derived branches of haplotypes, distantly related to the dominant sequence. Within these, the frequency of isolates derived from canids was 7.4% (7/95), which was much lower than those from sheep (30.6%, 55/180) and humans (36.8%, 49/133). These seven canid isolates originated from Australia ($n = 3$), Asia (China, $n = 1$; Kazakhstan, $n = 1$), the Middle East (Iran, $n = 1$) and South America (Peru, $n = 1$).

The haplotype and nucleotide diversities for *E. granulosus* (s.s.) isolates from sheep and human origin were at least double those in canid definitive hosts (table 5). Furthermore, the rarefied allelic richness of *E. granulosus* derived from canids was also approximately half that shown in sheep or humans, suggesting that these differences in genetic diversity are not purely a reflection of sample size. Tajima's D values were significantly negative for *E. granulosus* (s.s.) derived from all three hosts, indicating the presence of rare polymorphic sites characteristic of population expansion. The values of Fu's Fs for *E. granulosus* populations from sheep and humans were twice as high as those from canids, and deviated significantly from neutrality for all host species, also indicating population expansion. The values of the pairwise comparison for genetic differentiation (Fst) of *E. granulosus* (s.s.) between host species were generally low, but comparisons with canid isolates were highly significant (canid/sheep, 0.02896, $P = 0.0008$; canid/human, 0.0359, $P = 0.0002$; sheep/human, -0.0010, $P = 0.5186$).

Discussion

In the current study we analysed *cox1* mitochondrial sequences from *E. granulosus* (s.s.) adult tapeworm isolates recovered from dog, dingo, foxhound and jackal (*C. aureus*) definitive hosts ($n = 87$) and compared them to published sequence data for the metacestode stage from sheep ($n = 180$), humans ($n = 133$) and dogs ($n = 8$). The occurrence of *E. granulosus* (s.s.) in canid definitive hosts (dog, red fox, dingo, jackal, wolf) from many locations worldwide has been confirmed via molecular genetic analysis by several researchers (reviewed by Carmena & Cardona, 2013; Jenkins *et al.*, 2014). However, the current study reports the first molecular confirmation of *E. granulosus* (s.s.) from definitive hosts originating from Gaza and the Falkland Islands. This is also the first attempt to investigate the global genetic variation and population structure of *E. granulosus* (s.s.) isolates from definitive hosts, although this subject has been explored extensively for the metacestode stage of *E. granulosus* derived from ungulate and human hosts (Nakao *et al.*, 2010; Casulli *et al.*, 2012; Yanagida *et al.*, 2012; Sharma *et al.*, 2013; Boufana *et al.*, 2014, 2015).

The haplotype network generated using *cox1* mitochondrial sequenced data for *E. granulosus* (s.s.) DNA derived from definitive hosts is consistent with that previously described for the metacestode stage retrieved from sheep and human hosts (Nakao *et al.*, 2010; Casulli *et al.*, 2012; Yanagida *et al.*, 2012; Boufana *et al.*, 2014, 2015) with the dominance of the same *E. granulosus* centrally positioned haplotype. This finding therefore supports the worldwide common lineage of *E. granulosus* in canids from all geographical regions studied (i.e. Kenya, Libya, Tunisia, Australia, China, Kazakhstan, UK, Gaza, Peru and the Falkland Islands). It also confirms the absence of phylogeographic structure for *E. granulosus* (s.s.), as previously reported (Nakao *et al.*, 2010). In addition, the very low Fst values observed across this study suggest

Table 5. Diversity and neutrality indices for *Echinococcus granulosus* (s.s.) isolates from canid, sheep and human hosts using nucleotide data of the cytochrome *c* oxidase subunit 1 (*cox1*) mitochondrial gene.

Host (n)	hn	hd ± SD	πd ± SD	Rs	Tajima's D	P value	Fu's Fs	P value
Canid (95)	12	0.3592 ± 0.0639	0.001654 ± 0.001527	11.000	−1.9737	0.0022*	−11.5198	0.0000**
Sheep (180)	31	0.6919 ± 0.0358	0.003892 ± 0.002719	19.455	−2.0663	0.0011*	−28.7479	0.0000**
Human (133)	29	0.7787 ± 0.0342	0.004533 ± 0.003050	22.607	−2.1295	0.0017*	−27.9899	0.0000**

n, number of isolates; hn, number of haplotypes; hd, haplotype diversity; πd, nucleotide diversity; SD, standard deviation. Rs, rarefied allelic richness, standardized to the smallest sample collection ($n = 95$). Significant at *P value ≤ 0.01, **P value ≤ 0.0001.

the absence of genetic differentiation between adult *E. granulosus* isolates, which is consistent with that reported for isolates of the metacestode stage from ungulates and humans from China (Nakao *et al.*, 2010), Europe (Casulli *et al.*, 2012) and the Middle East (Jordan, Iran) (Yanagida *et al.*, 2012), but differs from that reported for Peruvian metacestode populations, which were said to be genetically differentiated from *E. granulosus* populations from China and the Middle East (Yanagida *et al.*, 2012).

A combination of higher haplotype and lower nucleotide diversities, similar to those reported for *cox1* nucleotide data derived from the metacestode stage of *E. granulosus* (Nakao *et al.*, 2010; Casulli *et al.*, 2012; Yanagida *et al.*, 2012; Boufana *et al.*, 2014, 2015), was seen in this study. The overall negative values for the neutrality indices (Tajma's *D* and Fu's Fs) observed in this study for adult *E. granulosus* (s.s.) were similarly reported for the larval metacestode stage, and signify populations under expansion. Conversely, the positive neutrality indices for the Australian *E. granulosus* samples indicate intermediate frequency of variant alleles. Additionally, comparatively low haplotypic variation was seen in this study for isolates from Australia and the UK, which may be due to the geographical isolation of these populations and would suggest moderate to limited sharing of haplotypes/alleles. In a recent study, a 'mainland–island' hypothesis was suggested to explain the higher genetic diversity in *Echinococcus multilocularis* populations from central Europe as compared to those from peripheral regions (Davidson *et al.*, 2012). However, segregated populations may still share haplotypes as a result of their shared ancestral history, and this was observed in the current study with the occurrence of the main *E. granulosus* haplotype (EgCN04) in the Australian and UK populations of *E. granulosus* (s.s.). No similar deductions could be drawn robustly for isolates from Asia and South America, due to their small sample size. We also used publically available sequence data to compare genealogical relationships between *E. granulosus* by host (95 canid, 180 ungulate, 133 human). The resultant network showed the presence of the common *E. granulosus* haplotype and the neutrality indices were significantly negative, indicating population expansion and the presence of rare haplotypes.

The interesting finding of this work is the decreased genetic variation exemplified by the low haplotype and nucleotide diversities recorded for the *cox1* mitochondrial nucleotide sequences derived from adult worms as compared to those from the metacestode stage from sheep and humans. *Echinococcus granulosus* is a hermaphrodite organism with predominant self-fertilization occurring in the intestine of the definitive host (Haag *et al.*, 1999; Nakao *et al.*, 2010) and massive asexual reproduction in the intermediate mammalian host. Self-fertilization is known to promote homozygosity (Lymbery *et al.*, 1997) and as a result genetic variation decreases. In this study we examined genetic variation using a maternally inherited gene (*cox1*) and thus the observed loss of heterozygosity may not be related to the occurrence of inbreeding, as no recombination is known to occur in mitochondrial genes. According to Lymbery *et al.* (1997) self-fertilization of *Echinococcus* occurs through autogamy and geitonogamy, and both these processes are thought to be responsible for the increase in homozygosity within *Echinococcus* populations (Nakao *et al.*, 2003). This is consistent with the low haplotype variation observed in the current study for *E. granulosus* (s.s.) adult tapeworms derived from canid hosts. Additionally, the discrepancies in haplotypic variation recorded in this study may be due to the relatively short lifespan of the adult worm in canid definitive hosts (<1 year) as compared to the lifespan of the metacestode stage, which is usually related to that of the host (sheep/human) and can last for several years (Gemmell, 1990). This would suggest that the reservoirs of genetic variation within *E. granulosus* in wild populations are the intermediate rather than the definitive canid hosts. However, to evaluate this further the examination of a larger number of canid hosts over a longer time period is required.

In summary, the current study compared DNA sequence data for the *cox1* gene from adult *E. granulosus* (s.s.) derived from definitive hosts and indicated a much more restricted haplotype profile compared to that published for intermediate (ungulates) and human hosts. It has also been suggested that the biphasic reproduction of *E. granulosus* may promote reduced genetic variability, even in nuclear genes (Nakao *et al.*, 2010). Improved nuclear single-locus genetic markers are needed to investigate further the differences in haplotypic diversities observed in this study.

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Conflict of interest

None.

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