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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers which are referred to in the text by their Roman numerals. The papers listed have been reproduced with permission of the copyright owners.

I. **Kinkar, L.**, Laurimäe, T., Simsek, S., Balkaya, I., Casulli, A., Manfredi, M.T., Ponce-Gordo, F., Varcasia, A., Lavikainen, A., González, L.M., Rehbein, S., van der Giessen, J., Sprong, H., Saarma, U. (2016). High-resolution phylogeography of zoonotic tapeworm *Echinococcus granulosus* sensu stricto genotype G1 with an emphasis on its distribution in Turkey, Italy and Spain. ***Parasitology***, 143, 1790–1801, © Cambridge University Press. DOI: 10.1017/S0031182016001530

II. **Kinkar, L.**, Laurimäe, T., Sharbatkhori, M., Mirhendi, H., Kia, E.B., Ponce-Gordo, F., Andresiuk, V., Simsek, S., Lavikainen, A., Irshadullah, M., Umhang, G., Oudni-M'rad, M., Acosta-Jamett, G., Rehbein, S., Saarma, U. (2017). New mitogenome and nuclear evidence on the phylogeny and taxonomy of the highly zoonotic tapeworm *Echinococcus granulosus* sensu stricto. ***Infection, Genetics and Evolution***, 52, 52–58, © Elsevier. DOI: 10.1016/j.meegid.2017.04.023

III. **Kinkar, L.**, Laurimäe, T., Acosta-Jamett, G., Andresiuk, V., Balkaya, I., Casulli, A., Gasser, R.B., van der Giessen, J., González, L.M., Haag, K.L., Zait, H., Irshadullah, M., Jabbar, A., Jenkins, D.J., Kia, E.B., Manfredi, M.T., Mirhendi, H., M'rad, S., Rostami-Nejad, M., Oudni-M'rad, M., Pierangeli, N.B., Ponce-Gordo, F., Rehbein, S., Sharbatkhori, M., Simsek, S., Soriano, S.V., Sprong, H., Šnábel, V., Umhang, G., Varcasia, A., Saarma, U. (2018). Global phylogeography and genetic diversity of the zoonotic tapeworm *Echinococcus granulosus* sensu stricto genotype G1. ***International Journal for Parasitology***, © Elsevier. DOI: 10.1016/j.ijpara.2018.03.006

IV. **Kinkar, L.**, Laurimäe, T., Balkaya, I., Casulli, A., Zait, H., Irshadullah, M., Sharbatkhori, M., Mirhendi, H., Rostami-Nejad, M., Ponce-Gordo, F., Rehbein, S., Kia, E.B., Simsek, S., Šnábel, V., Umhang, G., Varcasia, A., Saarma, U. (2018). Genetic diversity and phylogeography of the elusive, but epidemiologically important *Echinococcus granulosus* sensu stricto genotype G3. ***Parasitology,*** © Cambridge University Press. DOI: 10.1017/S0031182018000549

My personal contribution to the articles referred to in the thesis is as follows:
I–IV responsible for laboratory procedures, data analyses and manuscript preparation.

1. INTRODUCTION

Flukes (*Trematoda*), roundworms (*Nematoda*) and tapeworms (*Cestoda*) constitute the three major groups of helminths that parasitize humans and other animals, representing an enormous health and economic burden globally (Hotez et al., 2008). Helminths are particularly widespread in low-income regions of the world – it is estimated that over one billion people in developing regions of Asia, sub-Saharan Africa and the Americas are infected with one or more parasitic worm species (WHO, 2012). Helminths can be transmitted to humans through contaminated soil, food and/or water, but also through arthropod and molluscan vectors. The worms can infect every organ and their effects on the host species may vary from mild to deadly (Lindquist and Cross, 2017).

Tapeworms are flat, segmented worms, comprising species of a few millimetres (*Echinococcus* spp) up to several metres in length (*Diphyllobothrium* and *Taenia* spp). Albeit tiny, tapeworms of the genus *Echinococcus* cause a life-threatening zoonotic disease called echinococcosis. Echinococcosis has a long history dating back to antiquity, as the first indications of this disease stem from Hippocrates (~460–377 BP) (Eckert and Thompson, 2017). Nevertheless, the disease is still relevant, having a significant socioeconomic impact to this day.

The genus *Echinococcus* Rudolphi, 1801 (Cestoda: Taeniidae) comprises several species which cause echinococcosis in three forms: cystic echinococcosis, caused by *E. granulosus* sensu lato (s. l.), alveolar echinococcosis (*E. multilocularis*) and polycystic echinococcosis (*E. oligarthra* and *E. vogeli*). The two forms of public health relevance are cystic echinococcosis (CE) and alveolar echinococcosis (AE). Polycystic echinococcosis is less frequent and restricted to South and Central America (Tappe et al., 2008). *Echinococcus granulosus* s. l. and *E. multilocularis* are ranked 2nd and 3rd, respectively, in the list of food-borne parasites globally, while both CE and AE are considered among the 17 Neglected Tropical Diseases (NTDs) prioritized by the World Health Organization (FAO/WHO, 2014; WHO, 2015; Budke et al., 2017). The diseases are considered 'neglected' as they rank low on the priorities of governments and public health communities. Some of the other diseases listed among NTDs include leishmaniases, rabies, schistosomiasis and soil-transmitted helminthiases (WHO, 2015).

Echinococcus multilocularis is widely distributed in the northern hemisphere and is typically maintained in a sylvatic lifecycle including canids and various species of rodents, while *E. granulosus* s. l. has a cosmopolitan distribution and infects a wide range of both wild and domestic animals (Deplazes et al., 2017). Thus, CE is not only a substantial human health problem, but represents a considerable economic burden on livestock industries. It has been estimated that approximately one million or more people are suffering from CE globally, while the disease causes monetary losses of up to 2 billion US dollars in global livestock industry annually (Torgerson and Macpherson, 2011).

1.1. Lifecycle of *Echinococcus granulosus* sensu lato (s. l.)

The adult worm of *E. granulosus* s. l. is a few millimeters long (2–7 mm) and the mature worm possesses up to 5–6 segments, rarely more. The attachment organ is called a scolex and has two rows of hooks and four muscular suckers. The adult worm is a hermaphrodite and reproduces sexually, either by selfing or cross-fertilization, whereas the larval metacestode proliferates asexually (Eckert et al., 2001; Thompson, 2017).

Echinococcus granulosus s. l. has a life cycle involving two hosts: a carnivorous definitive host, which harbors adult worms, and a herbivorous or omnivorous intermediate host, in which the larval stage in the form of hydatid cysts develops. The parasite has an exceptionally wide host spectra, including mainly wild and domesticated ungulates, but also marsupials and primates as intermediate hosts, and various species of canids as definitive hosts. The hydatid cysts are fluid-filled structures in which up to thousands of protoscoleces are produced, each capable of developing into an adult worm in the definitive host (Thompson, 2017). The lifecycle of the parasite requires a predator-prey relationship, as the definitive host acquires the infection by consuming the infected organs of prey animals. Adult worms in the definitive hosts produce eggs, containing embryos (oncospheres) which are shed into the environment with faeces, subsequently ingested by a suitable herbivorous or omnivorous host (Eckert et al., 2001; Thompson, 2017) (Fig. 1). The eggs are covered by a highly resistant outer layer, and are thus able to survive up to several months in a suitable humid environment, but are sensitive to desiccation (Eckert et al., 2001; Eckert and Deplazes, 2004).

Humans are considered aberrant intermediate hosts of the parasite in which the larval stage develops. Cysts develop in various organs, most commonly liver (~75%) and lungs (~22%), but infections in muscles, kidneys, brain, spleen and other sites also occur (Eckert et al., 2001). Humans acquire the infection by accident, most commonly through close contact with dogs, as eggs can adhere to the coat of the animal. Other routes of transmission include the consumption of contaminated food (vegetables, salads, fruits and other plants) and water or handling egg-containing faeces or soil (Eckert and Deplazes, 2004; Deplazes et al., 2011). Although CE has a long asymptomatic incubation period that can last several years, severe clinical symptoms can be induced by cysts that have reached a particular size. Symptoms include abdominal pain, fever, vomiting, rashes, chest pain, chronic cough or shortness of breath. The most common methods of treatment are antiparasitic drugs, surgery or percutaneous techniques and if left untreated, CE can be life-threatening (Brunetti et al., 2010; Kern et al., 2017).

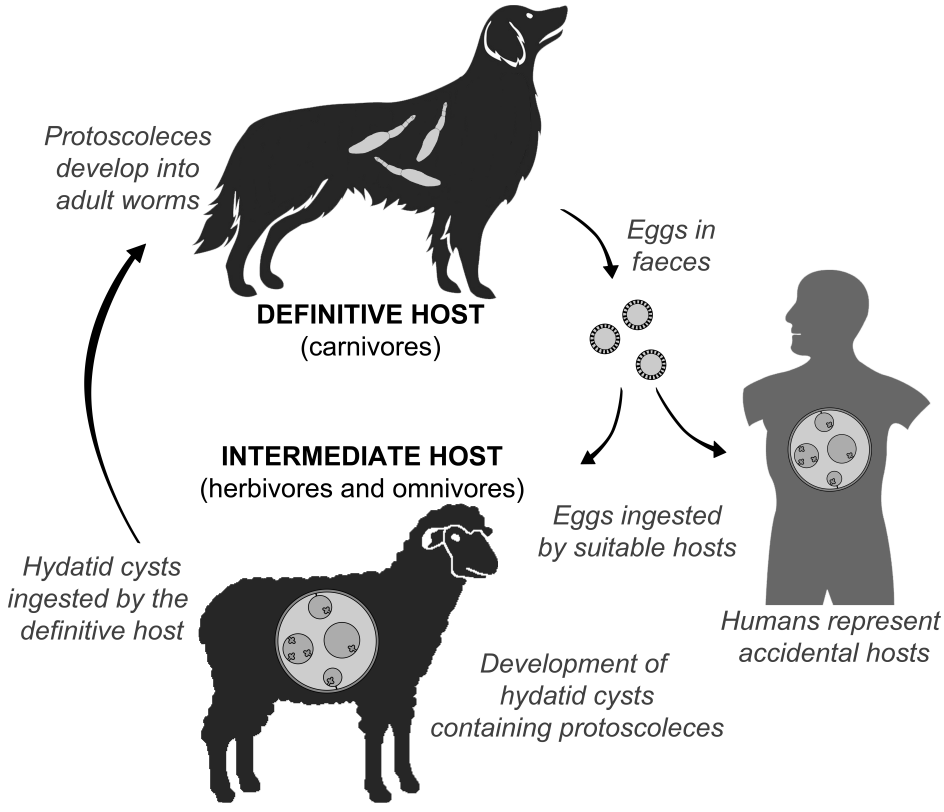

Figure 1. Lifecycle of *Echinococcus granulosus* sensu lato. Definitive hosts include several species of canids (e.g., dogs, wolves, jackals, dingoes), while intermediate hosts include a wide range of wild and domesticated species of mammals (e.g., sheep, cattle, goat, pig, buffalo, wild boar, moose, reindeer, wallaby, kangaroo). Humans represent accidental intermediate hosts.

1.2. Genotypes and species of *E. granulosus* s. l.

The taxonomy of *E. granulosus* s. l. has been a topic of controversy for decades. While species and strains were initially characterized based on differences in morphology, host occurrence, geographic distribution, and developmental biology, molecular studies based on mitochondrial (mtDNA) and nuclear DNA (nDNA) have clarified the extent of genetic variation and phylogenetic relations within *E. granulosus* s. l. (Lymbery, 2017). It is now regarded as a species complex as a number of genotypes ('strains') and species have now been characterized. Initially, 10 genotypes were identified (G1–G10), however, G9 is no longer considered a valid genotype and it has been speculated that G2 could also be invalid and represents a variant of G3 (Bowles et al., 1992, 1994; Scott et al., 1997; Thompson and McManus, 2002; Lavikainen et al., 2003; Vural et al., 2008; Abushhewa et al., 2010). Suggestions have been made to split these

genotypes into distinct species: *E. granulosus* s. s. (G1–G3), *E. equinus* (G4), *E. ortleppi* (G5) and *E. canadensis* (G6–G8, G10) or *E. intermedius* (G6, G7) and *E. canadensis* (G8, G10) (Thompson and McManus, 2002; Moks et al., 2008; Thompson, 2008; Saarma et al., 2009; Knapp et al., 2011, 2015; Lymbery et al., 2015; Nakao et al., 2015; Yanagida et al., 2017; Laurimäe et al., 2018a). In addition, the species *E. felidis* is now also considered to belong to *E. granulosus* s. l. (Hüttner et al., 2008). However, the taxonomy is still under dispute. For example, a study by Yanagida et al. (2017) used two nuclear loci and suggested the sharing of nuclear alleles between genotypic groups G6/G7 and G8/G10, whereas recent data based on six nuclear loci suggested that G6/G7 and G8/G10 are two distinct species (Laurimäe et al., 2018a). In addition, the evidence to regard *E. granulosus* s. s. as a single species is inconclusive as taxonomic studies of nuclear loci have never explicitly included G2 and G3.

Although extensive research has been carried out to understand the extent of genetic diversity of *E. granulosus* s. l., recent studies have highlighted that our knowledge remains incomplete as new highly divergent haplotypes within this complex have been characterized (Wassermann et al., 2016; Laurimäe et al., 2018b).

1.3. Distribution and host spectra of *E. granulosus* sensu stricto (s. s.)

Echinococcus granulosus s. s. is the most widespread species of *E. granulosus* s. l. and also the most frequent causative agent of CE of humans (88% of sequenced cases; Alvarez Rojas et al., 2014) and thus deserves particular attention. The species is spread worldwide, while highly endemic foci exist in South America, the Mediterranean Basin and Asia where poorer communities of rural livestock-raising areas are most affected (Jenkins et al., 2005; Dakkak, 2010; Jabbar et al., 2011; Hajialilo et al., 2012; Cardona and Carmena, 2013; Alvarez Rojas et al., 2014; Rostami et al., 2015; Cucher et al., 2016). Some of the main factors contributing to the persistence of CE include the frequent illegal and home slaughtering of animals for food, feeding raw offal to dogs, low public awareness of the disease, large populations of stray dogs and poor hygiene conditions (Eckert et al., 2001; Torgerson and Budke, 2003; Varcasia et al., 2011; Possenti et al., 2016).

Of the three genotypes characterized within *E. granulosus* s. s. (G1–G3), G1 by far the most prevalent worldwide, especially in Africa, Australia, Southern Europe, South America and parts of Asia (e.g., Breyer et al., 2004; Bart et al., 2006; Varcasia et al., 2007; Šnabel et al., 2009; de la Rue et al., 2011; Addy et al., 2012; Pezeshki et al., 2013; Alvarez Rojas et al., 2016). While relatively few cases of G3 have been reported in South America, Australia and North Africa (e.g., M'rad et al., 2010; de la Rue et al., 2011; Espinoza et al., 2014; Alvarez Rojas et al., 2016; Zait et al., 2016), significantly higher prevalence is charac-

teristic to Iran, Italy, Pakistan, Serbia and especially India (e.g., Capuano et al., 2006; Busi et al., 2007; Pednekar et al., 2009; Latif et al., 2010; Sharbatkhori et al., 2011; Sharma et al., 2013a, 2013b; Debeljak et al., 2016; Ehsan et al., 2017). G2 is the least prevalent genotype of *E. granulosus* s. s. and few cases have generally been reported worldwide (e.g., Kamenetzky et al., 2002; Guo et al., 2011; Casulli et al., 2012).

Of all *E. granulosus* s. l. species, *Echinococcus granulosus* s. s. has the widest host spectra including domestic and wild ungulates (e.g., sheep, cattle, goat, pig, buffalo, wild boar), marsupials, camelids and several other mammals as intermediate hosts and primarily dogs, but also jackals, wolves and dingos as definitive hosts (Romig et al., 2017). The parasite perpetuates primarily in a domestic lifecycle, while the most important and widespread cycle involves dogs and sheep (Cardona and Carmena, 2013). Although G1–G3 have a largely overlapping host spectra, G1 has the widest host range of the three genotypes (Thompson, 2017).

1.4. Molecular characterization and genetic diversity of *E. granulosus* s. s.

Genotypes G1–G3 were first molecularly defined based on short fragments of the mtDNA *cox1* (366 basepairs; bp) and *nad1* (471 bp) genes (Bowles et al., 1992; Bowles and McManus, 1993). The partial *cox1* and *nad1* mtDNA sequences have provided the basis for *E. granulosus* s. l. genotype distinction and the markers have represented highly valuable tools to investigate the genetic diversity and distribution of *E. granulosus* s. l. genotypes. According to the originally published sequences, G1–G3 differ by 1–3 positions in the *cox1* or *nad1* gene regions.

Genotype identification and research on the genetic diversity and phylogeography of *E. granulosus* s. s. has most commonly been based on the same few hundred bp fragments of the *cox1* and *nad1* genes, rarely longer sequences (e.g., 1609 bp of the *cox1* gene). After decades of research, it became increasingly evident that the genetic variation is significantly higher than initially characterized, and accumulating data identified a large proportion of haplotypes not homologous with any of the sequences of G1, G2 or G3 originally described in Bowles et al. (1992), but that clearly belong to the same cluster (e.g., Vural et al., 2008; Šnabel et al., 2009; Casulli et al., 2012; Yanagida et al., 2012; Andresiuk et al., 2013; Romig et al., 2015). In addition to the high intragenotypic variation, low intergenotypic variation between G1–G3 has also been demonstrated (e.g., Casulli et al., 2012; Andresiuk et al., 2013; Romig et al., 2015). These two pressing issues are especially well highlighted in a phylogenetic network of 137 *E. granulosus* s. s. haplotypes in Romig et al. (2015), based on 1609 bp of the *cox1* gene. The phylogenetic network revealed a low level of differentiation into genotypes G1, G2 and G3, without clear differen-

tiation into separate haplogroups. Furthermore, a large proportion of the haplotypes were not homologous with the sequences originally characterized in Bowles et al. (1992). Thus, the allocation of samples to G1–G3 has been dubious and without a clear definition, and the rationale of distinguishing these genotypes has been questioned.

Despite the ambiguity in the definition of the genotypes, numerous studies have been carried out that have significantly contributed to our knowledge on the genetic diversity and population structure of *E. granulosus* s. s. (e.g., Nakao et al., 2010; Casulli et al., 2012; Rostami Nejad et al., 2012; Yanagida et al., 2012; Andresiuk et al., 2013; Yan et al., 2013; Boufana et al., 2014, 2015; Alvarez Rojas et al., 2016, 2017; Hassan et al., 2017). The majority of the phylogenetic networks constructed thus far have yielded star-like structures with a commonly identified dominant central haplotype highly prevalent worldwide (e.g., Nakao et al., 2010; Casulli et al., 2012; Yanagida et al., 2012; Boufana et al., 2014, 2015). This common haplotype has been considered a founder lineage with a common source, from where a subsequent expansion of this species originated. It has been hypothesized that the Middle East is a possible candidate for the origin of *E. granulosus* s. s., as the genetic diversity in this region is higher than in several others (Yanagida et al., 2012). However, these hypotheses are awaiting further research.

1.5. Aims of the thesis

Despite the extensive research carried out on the inter- and intragenotypic genetic structure of *E. granulosus* s. s., significant gaps in knowledge still exist. The relatively short mtDNA sequences used so far (up to 1609 bp, whereas the full mtDNA of this species is ~13 500 bp), have yielded low resolution on phylogenetic networks and thus, the full extent of the mtDNA genetic variation within *E. granulosus* s. s. has remained unexplored, hindering detailed analyses of the taxonomy, genetic structure and phylogeographic history of this genotypic group.

Firstly, one of the most pressing issues is the existence and distinction of *E. granulosus* s. s. mitochondrial genotypes. Although the analysis of the 1609 bp *cox1* gene sequences demonstrated that G1–G3 are nearly inseparable on the phylogenetic network and the rationale of distinguishing these genotypes in the future has been questioned, the distinction and genetic distance of G1–G3 based on significantly longer mtDNA sequences, has remained unexplored. This is particularly important to elucidate, as this information underpins our fundamental understanding of the genetic make-up of *E. granulosus* s. s., the most commonly associated species of human echinococcosis.

Secondly, although after the initial molecular characterization of genotypes G1–G3 in the beginning of the 1990s, a proposal was made to treat G1–G3 as a single species due to their high genetic similarity based on mtDNA data, the evidence is still inconclusive. The taxonomic studies of nuclear loci have never

explicitly included all of the mitochondrial genotypes of *E. granulosus* s. s in analyses. However, this is crucial, as it would provide means to investigate the exchange of genetic material between the genotypes. Thus, despite the assumptions that the mitochondrial genotypes can be regarded as a distinct species *E. granulosus s. s.*, further analysis is required.

Thirdly, fascinating hypotheses have been proposed based on the phylogeographic studies on *E. granulosus* s. s. so far. Yet, due to the relatively short sequences used so far, the analyses have lacked sufficient phylogenetic power to reveal detailed insight into the phylogeographic history of the parasite. Also, the research so far has mostly included local populations, but there has been no global study. In addition, due to the ambiguity in the genetic differentiation of G1–G3, no studies so far have attempted to analyse the patterns of genetic diversity separately for the *E. granulosus* s. s. genotypes, thus possibly revealing differences in their phylogeographic history.

The present thesis aims to fill these gaps in our knowledge and the specific objectives were as follows:

(i) to assess the existence and distinction of *E. granulosus* s. s. mitochondrial genotypes G1–G3 using near-complete mtDNA sequences and a large panel of globally distributed samples (Kinkar et al., 2017, **II**; Kinkar et al., 2018a, **III**; Kinkar et al., 2018b, **IV**),

(ii) to analyse the taxonomic status of this genotypic group using sequence data of several nuclear loci for all genotypes of *E. granulosus* s. s. (**II**),

(iii) to provide detailed insight into the global patterns of genetic diversity and phylogeography of all *E. granulosus* s. s. genotypes, analysing near-complete mtDNA sequences of a large panel of globally distributed samples and highlight the advantage of using long sequences of mtDNA instead of the commonly used shorter sequences (Kinkar et al., 2016, **I**; **III**; **IV**).

2. MATERIALS AND METHODS

2.1. Parasite material

A total of 293 globally distributed *E. granulosus* s. s. (G1 and G3) samples obtained from various host species (sheep, cattle, human, wild boar, domestic pig, goat, buffalo, camel, dingo), one *E. equinus* (G4) sample from a Turkish donkey and three *E. ortleppi* (G5) samples from Indian buffaloes were analysed in this study (see Table 1 in **I–IV**; Fig. 1 in **I–IV** and Fig. 2 in **III)**. In addition, one genotype G1 sequence originating from China was obtained from GenBank (AB786664; Nakao et al., 2013). The samples sequenced in the present study were obtained during routine parasite inspection or from hospital cases and were ethanol-preserved at –20 ^{o}C until further use.

2.2. DNA extraction, PCR amplification, sequencing and assembly

DNA extraction
DNA was extracted from protoscoleces, cyst membranes or adult worms using High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany), following the manufacturer's protocols.

PCR amplification, sequencing and assembly of mtDNA
For PCR amplification of the mitogenome, 27 novel primers were designed (see Table 2 in **I** and **II**). Of these, 20 were used to amplify 8269–8274 bp of mtDNA (**I**) and 24 were used to amplify near-complete mitogenome sequences of 11 442–11 678 bp (**II–IV**). Sequencing was performed using the same primers as for the initial PCR amplification. For PCR cycle parameters and sequencing conditions, the reader is referred to the Materials and methods section '*DNA extraction, PCR amplification and sequencing*' in paper **I**. Sequences were assembled in CodonCode v4.2.7 (**I**), v.6.0.2 (**II–IV**) and manually curated in BioEdit v7.2.5 (Hall, 1999). All sequences were deposited in GenBank and are available under accession numbers KU925351–KU925433 (**I**), KY766882–KY766908 (**II**), MG672124–MG672293 (**III**) and MG682511–MG682544 (**IV**).

PCR amplification, sequencing and assembly of nuclear DNA
Amplification and sequencing of 3 nuclear genes in paper **II** (2984 bp in total): transforming growth factor beta receptor kinase (*tgf*; 937 bp), calreticulin (*cal*; 1272 bp) and elongation factor 1 alpha (*ef1*; 775 bp) was carried out according to Saarma et al. (2009). Sequences were assembled in CodonCode v.6.0.2 and manually curated in BioEdit v7.2.5. All nuclear sequences were deposited in GenBank and are available under accession numbers KY766909–KY766920.

2.3. Datasets

To analyse the genetic variation and phylogeography of *E. granulosus* s. s. genotype G1 in Europe and to compare the phylogenetic resolution of different mitochondrial sequence lengths, article **I** represented 91 G1 samples originating from several European countries. To evaluate the taxonomy of *E. granulosus* s. s. and the mitochondrial distinction between genotypes G1 and G3, a total of 23 *E. granulosus* s. s. samples were included in paper **II**. In addition, one *E. equinus* (G4) and three *E. ortleppi* (G5) samples were included in this paper to evaluate the genetic distance between G1 and G3 in relation to the distance from other *E. granulosus* s. l. genotypes/species. The genetic diversity and large-scale phylogeographic patterns of genotypes G1 and G3 were analysed using 212 G1 samples (**III**) and 39 G3 samples (**IV**). Further analysis of the mitochondrial distinction between genotypes G1 and G3 using a significantly larger dataset than in paper **II**, was based on the combined G1 and G3 datasets in papers **III** and **IV**. Note that some samples overlapped in papers **I–IV**, hence the sum of samples analysed in these papers is larger than the total number of samples indicated in Section 2.1 (see Supplementary Table S1 in **III**, **IV** and S2 in **III**).

2.4. Data analyses

2.4.1. Phylogenetic analyses

Phylogenetic networks were calculated using Network v4.6.1.2 (**I, II**) and v4.6.1.5 (**III, IV**) (Bandelt et al., 1999) (http://www.fluxus-engineering.com, Fluxus Technology Ltd.), considering both indels and point mutations. In paper **I**, networks were constructed for 3 different alignments using the same set of samples (n = 91) but different sequence lengths: (i) 8274 bp of mtDNA; (ii) the full *cox1* gene of 1674 bp and (iii) 351 bp fragment of the *cox1* gene. In paper **II**, networks were calculated separately for the mtDNA and nuclear datasets. In paper **III**, networks were calculated for three sequence datasets: (i) 212 G1 and 10 G3 samples, (ii) sequences representing genotype G1 only (n = 212) and (iii) sequences representing genotype G1 from humans (n = 41). In paper **IV**, networks were calculated for three sequence datasets: (i) 212 G1 and 39 G3 samples, (ii) samples belonging to genotype G3 only (n = 39) and (iii) sequences representing human samples of G1 (n = 41; sequences from paper **III)** and G3 (n = 5).

The Bayesian phylogenetic analysis was performed for two different datasets. To assess the intragenotypic phylogenetic relations of genotype G1 and intergenotypic relations between genotypes G1 and G3, the first dataset represented altogether 222 *E. granulosus* s. s. samples, of which 212 belonged to genotype G1 and 10 to G3 (**III**). The second dataset represented 39 G3 samples in order to analyse the phylogenetic relations of genotype G3 (**IV**).

Both analyses were performed in the program BEAST 1.8.4 (Drummond et al., 2012) using BEAUti v.1.8.4 to generate the initial xml file for BEAST. For the first dataset (**III**), the general time-reversible nucleotide-substitution model with a proportion of invariable sites and gamma distributed rate variation (GTR+*I*+*G;* Tavaré, 1986; Gu et al., 1995) was used, while the Tamura-Nei nucleotide substitution model with gamma distributed rate variation (TRN+*G*) (Tamura and Nei, 1993; Yang, 1994) was used for the second dataset representing G3 samples only (**IV**). Both models of sequence evolution were determined using the program PartitionFinder 2.1.1 (Guindon et al., 2010; Lanfear et al., 2012, 2016). For both datasets, the exponential growth coalescent prior (Griffiths and Tavaré, 1994) was chosen for the tree, and a strict molecular clock was assumed owing to the intraspecific nature of the data (Drummond and Bouckaert, 2015). The posterior distribution of parameters was estimated by Markov Chain Monte Carlo (MCMC) sampling. MCMC chains were run for 10 million states, and sampled every 1000 states with 10% burn-in. Log files were analysed using the program Tracer v1.6 (Rambaut et al., 2014). Both trees were produced using TreeAnnotator v1.8.4 and displayed in FigTree v.1.4.3 (Rambaut, 2014).

2.4.2. Population indices

The population diversity indices – number of haplotypes (Hn), haplotype diversity (Hd) and nucleotide diversity (π) – were calculated using DnaSP v5.10.01 (**I, III, IV**) (Librado and Rozas, 2009). Neutrality indices Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997) (**I, III, IV**) and pairwise fixation index (Fst) (**I, III**) were calculated using the population genetics package Arlequin 3.1 (**I**), 3.5.2.2 (**III**, **IV**) (Excoffier et al., 2005). In paper **I**, indices were calculated for 3 datasets: (i) all G1 sequences (n = 91), (ii) different localities (Turkey, Spain, Italy and Southern Europe) and (iii) hosts (cattle and sheep). These datasets were calculated for three sequence lengths (8274 bp, 1674 bp and 351 bp). In paper **III**, indices were calculated for four different datasets: (i) all G1 sequences (n = 212); (ii) the three most numerous host species (cattle, sheep and human), (iii) five regions (South America, Africa, Asia/Australia, Europe and the Middle East), and (iv) eight countries for which sample size exceeded 10: Algeria, Argentina, Brazil, Iran, Italy, Spain, Tunisia and Turkey. In addition to datasets i–iv in paper **III**, the Fst value was also calculated between all G1 samples (n = 212) and G3 samples (n = 10). In paper **IV**, diversity and neutrality indices were calculated for one dataset representing all G3 samples (n = 39).

2.4.3. Bayesian phylogeographic analyses

The phylogeographic diffusion patterns of genotype G1 (**III**) and G3 (**IV**) were analysed using a Bayesian discrete phylogeographic approach (Lemey et al., 2009). This approach estimates ancestral locations from the set of sampled locations and annotates the discrete location states to tree nodes (Lemey et al., 2009; Faria et al., 2011). The standard Markov model was extended using a Bayesian Stochastic Search Variable Selection (BSSVS) procedure, which offers a Bayesian Factor (BF) test to identify the most parsimonious description of the phylogeographic diffusion process (Lemey et al., 2009). Specifically, the intial xml file generated in BEAUti in the Bayesian phylogenetic analysis was edited according to the 'Discrete phylogeographic analysis' tutorial available on the Beast website (http://beast.bio.ed.ac.uk/tutorials – accessed in June 2017). The analysis was performed in BEAST 1.8.4 (Drummond et al., 2012) using the BEAGLE library (Ayres et al., 2012). For the G1 dataset in paper **III**, MCMC chains were run for 50 million states, sampled every 5000 states with 10% burn-in. For the G3 dataset in paper **IV**, MCMC chains were run for 30 million states, sampled every 3000 states with 10% burn-in. The effective sampling size (ESS) of estimates was assessed using Tracer v1.6 (Rambaut et al., 2014), and the tree was produced using TreeAnnotator v1.8.4 and displayed in FigTree v.1.4.3 (Rambaut, 2014). The program SpreaD3 v0.9.6 (Bielejec et al., 2016) was used to visualize the output from the Bayesian phylogeographic analysis and to calculate the Bayes Factor supports. Three independent runs were conducted and geographic links that yielded an average value of BF > 10 were displayed.

3. RESULTS

3.1. Successfully analysed samples and sequences

In paper **I,** a total of 8274 bp of mtDNA was successfully sequenced for the 91 *E. granulosus* s. s. G1 samples (the length of alignment was 8274 bp, while sequence length varied between 8269–8274 bp).

In paper **II**, 23 *E. granulosus* s. s., one *E. equinus* and three *E. ortleppi* samples were successfully sequenced, yielding the final mtDNA alignment of 11 502 bp (the sequence length was 11 422–11 443 bp for the *E. granulosus* s. s. samples, 11 465 bp for the *E. equinus* sample and 11 466 for the *E. ortleppi* samples). Three G1 samples were the same as in paper **I**, but additional mitochondrial loci were sequenced (~3400 bp). Nuclear markers *cal*, *ef1* and *tgf* were also successfully PCR-amplified for the same set of samples, except for one sample that did not yield positive PCR results with the nuclear markers. The final length of the nuclear genes in alignment was 2984 bp.

Of the 221 samples sequenced in paper **III,** 114 were newly sequenced, whereas the rest were from papers **I**, **II** and from Laurimäe et al. (2016) (8279 bp; samples from South and Central America). However, additional mtDNA loci were sequenced for all of the overlapping samples (~3400 bp for the samples from paper **I** and Laurimäe et al. (2016) and ~240 bp for the samples from paper **II**). The 221 *E. granulosus* s. s. samples in paper **III** yielded an alignment of 11 682 bp. While most sequences were 11 675 bp in length, some varied from 11 674 bp to 11 678 bp.

In paper **IV**, a total of 39 *E. granulosus* s. s. G3 samples were successfully amplified, of which 27 were newly sequenced and 12 were from paper **II**. For these 12 samples, ~240 additional basepairs were sequenced. The final alignment of the G3 samples was 11 675 bp.

For further data on the overlapping sequences, see Supplementary Table S1 in **III**, **IV** and S2 in **III**.

3.2. Mitochondrial distinction between *E. equinus*, *E. ortleppi* and *E. granulosus* s. s.

The mtDNA haplotypes of *E. equinus* and *E. ortleppi* were genetically highly divergent from *E. granulosus* s. s., separated by 1244 and 1387 mutations, respectively (Fig. 2 in **II**). The genetic distance between *E. equinus* and *E. ortleppi* was 1228 mutations.

3.3. Mitochondrial distinction between *E. granulosus* s. s. genotypes G1 and G3, and the validity of G2

The *E. granulosus* s. s. samples were divided into two haplogroups, separated by 37 mutations (Fig. 2 in **II**, **IV** and Fig. 3 in **III**). The genotypes were designated according to the original genotype definitions sensu Bowles et al. (1992) (see section 3.1 in **II**, **III** and section '*The phylogenetic network of E. granulosus s. s.*' in **IV**). Therefore, these two haplogroups corresponded to the *E. granulosus* s. s. mitochondrial genotypes G1 and G3 and were named accordingly. The phylogenetic networks demonstrated that G1 and G3 are clearly distinct genotype groups in the context of mitochondrial data, as no sequences were positioned between G1 and G3.

The distinction of G1 and G3 was further supported by the Bayesian phylogenetic analysis which divided samples of genotype G1 and G3 into two well-supported clades (posterior probability value = 1.00; Figs. 4 and S1 in **III**) and the high Fst value between genotypes G1 and G3 (0.711; $p < 0.00001$ in **III**).

Of the 39 G3 samples analysed, altogether four corresponded to genotype G2 according to the original definition in Bowles et al. (1992). These four samples positioned inside the G3 cluster and were not monophyletic (Fig. 4 in **IV** and Fig. 2 in **II**).

3.4. Taxonomic status of *E. granulosus* s. s.

Data based on three nuclear genes demonstrated that in the taxonomic sense, G1 and G3 can be regarded as a single species *E. granulosus* s. s., as there was no clear separation between genotypes G1 and G3 based on the nuclear data.

The analysed 26 samples were divided into 4 distinct sequences (Fig. 3 in **II**). *Echinococcus granulosus* s. s. samples (n = 22; one sample did not yield positive PCR results with the nuclear markers) comprised 2 sequences, separated by a single mutation. One sequence was dominant, comprising 20 *E. granulosus* s. s. samples, whereas the other included 2 samples. The three analysed *E. ortleppi* samples had identical nuclear sequences, separated from *E. granulosus* s. s. by 36 mutations. The *E. equinus* sample was separated from *E. ortleppi* and *E. granulosus* s. s. by 23 and 45 mutations, respectively.

3.5. Genetic diversity and phylogeography of genotype G1 in Europe

3.5.1. Phylogenetic network

In the phylogenetic network, the analysed 91 sequences were divided into 83 haplotypes (Fig. 2 in **I**). No predominant haplotype was revealed in the phylogenetic network, most haplotypes were singletons (n = 76). Five haplotypes (TUR45, TUR10, TUR35, TUR56 and ITA3) included two samples and one haplotype (TUR3) included 4 samples.

Although geographically distant samples were often genetically distant (e.g., TUR41 from Turkey and SPA1 from Spain separated by 20 mutations) and geographically close samples genetically closely related (e.g., TUR11 and TUR13 from Turkey separated by 1 mutation), the opposite was also observed. Several samples obtained from geographically close localities showed remarkably high genetic distance (e.g., Turkish samples TUR12 and TUR26 both from Eastern Turkey separated by 24 mutations). In addition, numerous samples from different countries were frequently genetically closely related, as illustrated by several monophyletic groups that comprised closely-related samples from Turkey and other countries such as Albania, Greece, Romania and Spain. In addition, one group included an Italian (ITA4), Spanish (SPA7) and Finnish/ Algerian (FIN1) sample. No clear host-specific structure was detected in the phylogenetic network.

3.5.2. Diversity, neutrality and fixation indices

The overall haplotype diversity in Europe was 0.997, whereas nucleotide diversity was 0.00143 (Table 3 in **I**). Similar values were also observed in the Italian, Spanish and Turkish subpopulations, ranging from 0.952 to 1.000 and 0.00068 to 0.00147, respectively, while the Italian population showed slightly lower values for both indices. Haplotype diversity values for cattle and sheep were 0.999 and 0.991, while nucleotide diversities were 0.00152 and 0.00131, respectively.

Haplotype diversity was almost equally high for the 8274 bp (Hd = 0.997) and the full *cox1* gene datasets (1674 bp; Hd = 0.920; Table S1 in **I**), whereas considerably lower for the 351 bp dataset (Hd = 0.596; Table S2 in **I**). Nucleotide diversity increased with shorter sequences (Tables 3, S1 and S2 in **I**): based on the 8274 bp dataset, $\pi = 0.00143$, for the full *cox1* gene (1674 bp), $\pi = 0.00196$, and the value was 0.00219 for the partial *cox1* gene (351 bp). This indicates that the average diversity of the fragments of the *cox1* gene is higher compared with 8274 bp of mtDNA.

Neutrality indices such as Tajima's D and Fu's Fs were significant for most of the analysed datasets (Table 3 in **I**). The lowest negative values were detected for the overall population, Turkish samples and for cattle and sheep.

Low Fst values were observed among different localities (Table S3 in **I**). The Fst value for the 8274 bp dataset was statistically significant only between Spain and Turkey ($Fst = 0.04064$, $p < 0.05$). Relatively low Fst values ($Fst = 0.01180$, $p < 0.05$) were also recorded between cattle and sheep subpopulations.

3.5.3. Phylogenetic resolution in comparison with shorter mtDNA sequences

In the networks based on the reduced datasets of 1674 bp and 351 bp, sequences were divided into 49 and 11 haplotypes, respectively, of which two were predominant in both networks (Fig. 3 in **I**).

Longer sequences had significantly more power to reveal the genetic relations between different haplotypes. Not only were haplotypes fully resolved on the phylogenetic network based on long sequences, but in comparison between the 8274 bp and 1674 bp datasets, some haplotypes were positioned into different haplogroups (e.g., SPA7 and FIN1), whereas several haplotypes (e.g., SPA4, SPA10, TUR6, TUR9, TUR42 and TUR43) assumed different phylogenetic relations to each other (Figs. 2 and 3 in **I**). The 351 bp dataset positioned the majority of the samples into two central haplotypes (Fig. 3 in **I**).

3.6. Global genetic diversity, phylogeny and phylogeography of genotype G1

3.6.1. Phylogeny

The Bayesian phylogeny of genotype G1 yielded clades with varying support values, of which several clades were well resolved and received high posterior probability values (1.00; Figs. 4 and S1 in **III**).

The phylogenetic network for genotype G1 was highly divergent: the 212 G1 samples were divided into 171 haplotypes (Fig. 5 in **III**). Among the 171 haplotypes, 147 were represented by a single sample, 18 haplotypes included two samples, 5 haplotypes (IRA1, BRA1, TUR1, TUR3, TUN5) included 3 samples and one haplotype (ARB1) included 14 samples.

In the phylogenetic network, multiple haplogroups (i.e., monophyletic groups) could be distinguished. Seven haplogroups named A–G, respectively, corresponded to the well-supported clusters in the Bayesian phylogenetic tree (posterior probability values = 1.00; Figs. 4, 5 and S1 in **III**). Of the nine haplogroups in grey (Fig. 5 in **III**), seven were well-supported on the phylogenetic tree (posterior probability values = 1.00; Figs. 4 and S1 in **III**).

Some haplotypes in monophyletic clusters, grouped together according to geographic origin (Fig. 5 in **III**). For example, three monophyletic groups represented haplotypes only from Tunisia (TUN25, TUN11 and TUN1; TUN26

and TUN6; TUN13, TUN3 and TUN18). Another haplogroup (D) was of Middle Eastern origin, comprising samples from Turkey (TUR8, TUR21, TUR18, TUR19) and Iran (IRA11). In addition, one group was of North African origin and included samples from Tunisia (TUN5, TUN7) and Algeria (ALG9). Another group was of South American origin, including haplotypes from Brazil and Argentina (BRA4, ARG2, BRA6). In addition, haplogroup B included a central haplotype ARB1, which comprised samples from Argentina and Brazil. This haplogroup also included 12 haplotypes from Argentina, 4 haplotypes from Brazil (BRA7–BRA10), two haplotypes from Chile (CHI2 and CHI3) and one from Mexico (MEX1). In other monophyletic groups, samples from Eurasia clustered together, such as an Indian-Iranian group (IND1 and IRA16) and a Turkish-Spanish-Iranian group F (TUR12, TUR24, TUR27, TUR4, TUR9, IRA12 and SPA1). Haplogroup G from Eurasia represented haplotypes from Turkey (n = 12), Iran (n = 8), Albania (ALB1, ALB2), Moldova (MOL2) and Romania (ROM1), and haplogroup C represented haplotypes from Iran (IRA19, IRA6 and IRA5), Moldova (MOL3), Mongolia (MON1) and Romania (ROM2).

Some of the geographically most distant haplotypes that clustered together included two haplotypes from Australia (AUS1 and AUS2) and a haplotype originating from Algeria (ALG4) (Fig. 5 in **III**). Another haplotype from Australia (AUS3) clustered together with 12 haplotypes from Africa and three haplotypes from Europe (SPA7, SPA4 and FIN1; haplogroup A). In addition, five haplotypes from Africa (ALG2, TUN15, MOR1, TUN27, ALG8) grouped with haplotype ARG8 from Argentina, and haplotypes ITA7, ITA6, ITA8, and TUN2 from Italy and Tunisia clustered together.

The majority of monophyletic clusters included samples from different host species. The most numerous host species in this study, cattle and sheep, were genetically often closely related and some haplotypes (TUR17, TUN14 and ARB1) included samples from both hosts. The haplotypes representing 41 samples from humans did not cluster together and were positioned in different haplogroups, together with samples from other hosts. Haplotype TUN5 from Tunisia represented three samples, one from sheep and two from humans and haplotype TUN15 also from Tunisia represented two samples, one from sheep the other from a human.

3.6.2. Diversity, neutrality and fixation indices

The overall haplotype diversity index for genotype G1 was 0.994, while nucleotide diversity was 0.00133 (Table 2 in **III**). The most numerous host species in this study – cattle, sheep and human – were also represented by similar haplotype diversity values (0.987 to 0.995), whereas nucleotide diversities ranged from 0.00128 to 0.00138. The haplotype diversity indices for genotype G1 from the five geographical regions ranged from 0.923 to 0.994, whereas the nucleotide diversities varied from 0.00083 to 0.00136, with samples from South America having the lowest values. Argentina had the

lowest values of haplotype and nucleotide diversities (Hd = 0.832 and $\pi = 0.00057$), whilst the corresponding values for other countries were higher (ranging from 0.956 to 1.000 and π ranging from 0.115 to 0.00143).

Neutrality indices Tajima's D and Fu's Fs were negative and statistically highly significant for the whole G1 dataset ($D = -2.77$, Fs = –23.80; Table 2 in **III**). Neutrality indices were similar among host species and in the majority of the regions (Africa, South America, Europe and the Middle East). However, neutrality indices were statistically insignificant for Asia and Australia. Both neutrality indices were negative and statistically significant for Algeria, Argentina, Tunisia and Turkey, while only Tajima's D was significant for Iran. The neutrality indices calculated for Brazil, Italy and Spain were all negative, but statistically insignificant.

Low Fst values were observed between cattle, sheep and human samples of G1 (Fst < 0.05; Table 3 in **III**) and between most of the regions of G1 (Africa, Asia and Australia, Europe and the Middle East), ranging from 0.022 to 0.068 (Table 4 in **III**). However, higher Fst values (ranging from 0.184 to 0.213) were detected between South America and the other regions. The highest Fst values were between Argentina and the Eurasian (Iran, Italy, Spain and Turkey) and African countries (Algeria and Tunisia), ranging from 0.269 to 0.359, while the value was slightly lower between Argentina and Brazil (0.124; Table 5 in **III**). The Fst values between the remaining countries were mostly less than 0.100. Statistically insignificant values were observed between Europe and Asia-Australia (Table 4 in **III**) and between Algeria and Tunisia (Table 5 in **III**).

3.6.3. Bayesian phylogeographic analysis

The Bayesian phylogeographic analysis yielded 18 well-supported spatial diffusion routes for genotype G1, of which 11 had a Bayes Factor value of 10 to 100, whereas the BF value was very high (>100) for seven routes (Fig. 7 in **III**). Values of >3 are considered well-supported (Lemey et al., 2009). A total of seven routes originated from Turkey, two of which had very high support (BF > 100; between Turkey and Iran and Turkey and Greece); six originated from Tunisia, three of which had BF values >100 (between Tunisia and Italy, Tunisia – Algeria and Tunisia – Argentina). Argentina was the ancestral location to Brazil (BF > 100), Mexico and Chile, while Iran was ancestral to India. Algeria was identified as the origin of the sample from a human from Finland.

3.7. Genetic diversity, phylogeny and phylogeography of genotype G3

3.7.1. Phylogeny

The Bayesian phylogeny of genotype G3 revealed multiple clades with varying support values, of which six were well resolved and received high posterior probability values (≥0.9; Fig. 3 in **IV**).

The 39 G3 samples represented 34 distinct haplotypes in the phylogenetic network (Fig. 4 in **IV**). Among the 34 haplotypes, 30 were represented by a single sample, 3 haplotypes included two samples (TUR37, IND4 and FRA2) and SPA12 included 3 samples. Six haplogroups which corresponded to the well-supported clusters in the Bayesian phylogenetic tree (Fig. 3 in **IV**) could be distinguished and were named A–F, respectively (Figs. 3 and 4 in **IV**).

Samples of various geographic regions clustered together in the six haplogroups (Fig. 4 in **IV**). In groups D and B, Iranian samples grouped with some of the European samples (IRA20, ITA11 and IRA21, ALB3, GRE3) and in haplogroup F Iranian, Turkish and European samples clustered together (IRA25, TUR39, TUR43 and ROM3). While the majority of group E was represented by samples of European origin, an Algerian sample ALG13 also clustered into this haplogroup. Samples of Iranian and Indian origin comprised haplogroup A (IND3, IND4, IRA24 and IRA22).

While the majority of the monophyletic clusters included samples from different host species, group D was composed of two sheep samples. The three buffalo haplotypes in this study (IND3, IND4 and IND2) were most closely related to samples from camels (IRA24, IRA22 and IRA23) (see also haplogroup A) while in group F, a relatively divergent camel sample (IRA25) clustered together with samples from cattle (TUR39), sheep (TUR43) and human (ROM3). In haplogroups E and C, human samples of European (FIN2, BUL1 and SPA16) and Algerian (ALG13) origin clustered together with European sheep samples and in group B, two sheep samples (GRE3 and IRA21) grouped together with a sample from cattle (ALB3).

3.7.2. Diversity and neutrality indices

The overall haplotype diversity index for genotype G3 was 0.992 (S.D. ± 0.008), while nucleotide diversity was 0.00143 (S.D. ± 0.00007). Neutrality indices Tajima's D and Fu's Fs were negative and statistically significant ($D = -2.51$, $p < 0.000001$ and $Fs = -13.54$, $p < 0.01$).

3.7.3. Bayesian phylogeographic analysis

The Bayesian phylogeographic analysis yielded nine well-supported diffusion routes (BF > 10), of which two received the BF value of >100 (Fig. 5 in **IV**). These two strongly supported routes both originated from Turkey, suggesting a migration towards Romania and Iran. Iran was the ancestral location to India, Albania, Greece and Italy while Spain was ancestral to Algeria and Bulgaria (BF > 10). A well-supported diffusion route was also identified between Italy and France (BF > 10).

4. DISCUSSION

4.1. Distinction of *E. granulosus* s. s. genotypes based on mtDNA and nDNA data

The results based on near-complete mitochondrial genome sequences clearly demonstrate that G1 and G3 form distinct haplogroups, separated by 37 mutations in the phylogenetic network (Fig. 2 in **II**, **IV** and Fig. 3 in **III**). Previously, the most comprehensive analysis of the distinction of *E. granulosus* s. s. was assessed using the *cox1* gene (1609 bp), which yielded only 1–2 mutations between G1 and G3, without clear separation into distinct haplogroups (Romig et al., 2015). However, sequencing a significantly larger portion of the mitogenome in the present study (>11 400 bp) has allowed, for the first time, to demonstrate that G1 and G3 are, in fact, clearly distinct mitochondrial genotypes. This distinction was further supported by the Bayesian phylogenetic analysis (posterior probability value = 1.00; Figs. 4 and S1 in **III**) and by the high Fst value (0.711; $p < 0.00001$) between the two genotypes. It is important to note that the G1 and G3 samples were obtained not only from a wide geographical range, but also from countries where they exist in sympatry: Algeria, Albania, France, Finland, Greece, India, Iran, Italy, Romania, Spain and Turkey (Fig. 1 in **II**, **III** and **IV**). In addition, several host species analysed in the present study were shared between G1 and G3 (sheep, cattle, human and buffalo) (Table 1 in **II**, **III** and **IV**). Thus, the separation of these groups cannot be explained by clustering according to the geographical origin or host species of the samples. It is possible that future studies involving even larger datasets, may reveal haplotypes that position between G1 and G3 in mtDNA-based phylogenetic networks, as a few highly divergent haplotypes have been described within *E. granulosus* s. l. (Wassermann et al., 2016; Laurimäe et al., 2018b). Nevertheless, these cases are likely rare and since our analyses included samples from both geographically overlapping and highly distant locations, it can be concluded that genotypes G1 and G3 represent clearly distinct mitochondrial lineages.

The results of the mtDNA do not necessarily mean that genotypes G1 and G3 are separate biological entities. The mitochondrial genome does not undergo recombination and mutations accumulate at random. Once a mutation becomes fixed in a population, it forms a new lineage that is separate from the ancestral one. Mutations then continue to fix progressively in both the new and ancestral lineage in an independent manner. However, although mutations in the nDNA also accumulate at random, the nuclear genome does undergo recombination and in case of no barrier for gene flow, nuclear genes do not show separation into genetically distinct populations. Our data based on three nuclear genes distinguished *E. granulosus* s. s., *E. equinus* and *E. ortleppi* from each other with confidence, whereas there was no distinction between G1 and G3 (Fig. 3 in **II**), suggesting ongoing gene flow between the two genotypes. Thus, we were

able to confirm that in the taxonomic sense, G1 and G3 can be regarded as a single species *E. granulosus* s. s., which is further supported by the lack of ecological differences between the two genotypes.

Our data suggests that G2 is not a valid genotype. Altogether four samples, which corresponded to the original molecular definition of genotype G2 sensu 366 bp of *cox1* (Bowles et al., 1992), clustered together with the G3 samples in the mtDNA networks and with both G1 and G3 based on nuclear genes. In addition, the putative G2 haplotypes in the mtDNA network were not monophyletic (Fig. 2 in **II** and Fig. 4 in **IV**) and we therefore suggest excluding G2 from the genotype list.

As some *E. granulosus* s. l. genotypes are known to differ in terms of pathogenicity, infectivity, developmental rate, physiology and other aspects (Thompson, 2017), it is possible that relevant differences might occur between G1 and G3. Although this has not yet been explored between these two genotypes and remains to be studied in the future, applying up-to-date molecular diagnostics to reliably identify and distinguish between G1 and G3 is a crucial prerequisite to perform further research on this topic.

Although sequencing complete or near-complete mitogenomes is highly useful to gain deep insight into the genetic structure and phylogeography of this parasite, it might not be necessary for the assignment of samples into genotypes G1 or G3. Based on extensive sampling and sequencing data, we identified reliable diagnostic positions between G1 and G3 and developed a new genetic marker for the identification and distinction of the two genotypes (Kinkar et al., 2018c). We found that *nad5* is the best gene in mtDNA to differentiate between G1 and G3 as it offers clear advantages over the previous ones, providing a higher number of consistently diagnostic positions than the commonly used *cox1* and *nad1* genes.

4.2. Genetic diversity and structure of genotypes G1 and G3

Our results demonstrated very high global genetic variation within genotypes G1 and G3: the 212 G1 samples in paper **III** represented a total of 171 haplotypes, whereas the 39 G3 samples in paper **IV** divided into 34 haplotypes. The values of haplotype and nucleotide diversity were similar between G1 (Hd = 0.994; π = 0.00133) and G3 (Hd = 0.992; π = 0.00143), demonstrating that the genetic diversity is equally high for both genotypes, although G3 is globally significantly less prevalent than G1 (e.g., Breyer et al., 2004; Bart et al., 2006; Nakao et al., 2010; de la Rue et al., 2011; Casulli et al., 2012; Mitrea et al., 2014; Nikmanesh et al., 2014). Our results are in line with several previous studies reporting G3 to be less prevalent than G1: according to our combined datasets from papers **III** and **IV**, the prevalence of genotype G3 was 15.6% while all other *E. granulosus* s. s. belonged to G1 (84.4%). However,

this might not reflect the true global prevalence of G3, as some regions were underrepresented in the present thesis.

Due to the higher global prevalence of G1 and hence the significantly larger sample size of this genotype in the present study, we were able to further assess the patterns of global genetic diversity for genotype G1. Haplotype diversities within genotype G1 were high for different host species, most regions and countries (Table 3 in **I** and Table 2 in **III**), whereas Fst values were mostly low (Table S3 in **I** and Tables 3–5 in **III**). This points to high genetic diversity and low genetic differentiation between G1 subpopulations globally, particularly across the Mediterranean countries, as specifically addressed in paper **I**. The low genetic differentiation between subpopulations is further highlighted by the structure of the G1 phylogenetic network (Fig. 5 in **III**), where monophyletic clusters comprised samples from various geographic locations (e.g., haplogroup A, in which African, Australian and European samples clustered together). As the lifecycle of *E. granulosus* s. s. is maintained mainly by domestic animals, their distribution is subject to anthropogenic effects and thus these patterns are likely highly influenced by the extensive global animal transport and trade, resulting in the high degree of genetic diversity and lack of genetic differentiation between different regions. Although Fst values could not be calculated for different G3 subpopulations due to small sample size, similar patterns were also well-highlighted by the structure of the phylogenetic network of G3 (Fig. 4 in **IV**), where similarly to the G1 network, samples from various locations clustered together.

However, as highlighted in paper **III**, the South American samples (particularly Argentina) showed slightly lower values of haplotype diversities compared to other regions, coupled with higher values of Fst (Tables 2, 4 and 5 in **III**). This indicates lower genetic diversity and moderate genetic differentiation of samples from South America (particularly Argentina) compared with those from Africa and Eurasia. This is also supported by the structure of the phylogenetic network wherein some of the South American samples (and one sample from Mexico) formed a haplogroup with a dominant central haplotype comprising 14 Argentinian samples (Fig. 5 in **III**). A possible explanation for this is the more recent arrival to and sudden expansion of domestic animals (cattle and sheep) in South America during the 15th and 16th Centuries (Rodero et al., 1992) compared with the domestication history in Africa and Eurasia, extending thousands of years BC (Zeder, 2008; Lv et al., 2015). However, as Argentina contributed more to the lower Hd value for South America, this pattern could simply reflect the predominance of samples originating from the Buenos Aires province in Argentina (24 of 31) (Table S3 in **III**). However, the samples from Turkey in this study also originated from one area in the East (Erzurum and Elazig provinces), but yielded high haplotype diversity values nevertheless (Table 2 in **III**). Therefore, the results could indeed reflect a more recent arrival to and sudden expansion of *E. granulosus* s. s. genotype G1 in South America. However, to elucidate the genetic diversity and population

structure of the parasite in South America, further investigations are needed involving larger datasets.

By comparing networks drawn from different sequence lengths (8274 bp, 1674 bp and 351 bp; **I**), we were able to demonstrate that longer sequences revealed significantly higher resolution compared with the shorter sequences. One of the most striking differences was the dominance of the central haplotypes. Although all three networks revealed two central ancestral haplotypes, the number of samples that were positioned into the central haplotypes varied significantly. Both networks based on the shorter sequences (1674 bp and 351 bp) suggest that a wide geographical spectra of samples belong to both of the ancestral haplotypes, whereas these two haplotypes were fully resolved in the 8274 bp network (Figs. 2 and 3 in **I**). Furthermore, in both networks based on the shorter sequences, the most dominant haplotype was identical to haplotype EG1, which has been found to be highly prevalent worldwide (Nakao et al., 2010; Casulli et al., 2012; Yanagida et al., 2012; Boufana et al., 2014, 2015). However, the 8274 bp dataset showed that this haplotype is genetically highly diverse and was fully resolved. The networks also show that longer sequences have significantly more power to resolve the genetic relations compared with shorter sequences. For example, based on 8274 bp haplotypes SPA7 and FIN1 belonged to the haplogroup with the Italian central haplotype, whereas the network based on 1674 bp suggested that the two haplotypes belong to the other haplogroup with the Turkish central haplotype (Figs. 2 and 3 in **I**). Our results demonstrate that using longer mtDNA sequences for phylogenetic and -geographic analyses has indeed clear advantages over the commonly used shorter sequences. This has also been demonstrated for genotypes G6 and G7 in Laurimäe et al. (2018b), where complete mtDNA sequences were analysed revealing novel insight into the genetic structure of these genotypes. For example, genotype G7 was represented by two major haplogroups G7a and G7b, whereas sensu Bowles et al. (1992), the G7b samples would have been classified as genotype G6.

4.3. Phylogeographic history and divergence of genotypes G1 and G3

We performed the Bayesian phylogeographic analysis for the G1 (**III**) and G3 (**IV**) datasets. As an output, the analysis reconstructs hypothetical migration routes of these parasites on to a map. While these links could be highly influenced by the complex livestock transport circuits in relatively recent history, some of them seemed to follow the diffusion routes of livestock early in history. However, it should be emphasised that linking the well-supported diffusion routes to a timescale remains speculative. The analyses revealed a number of well-supported routes of genotypes G1 and G3 that seemed to follow the spread of livestock animals from the centre of domestication during

Neolithic times (Zeder, 2008; Lv et al., 2015) (Fig. 5 in **IV** and Fig. 7 in **III**). For both G1 and G3, well-supported diffusion routes from Turkey towards Southern Europe and Iran were revealed. Interestingly, while Turkey was the origin of a large-scale expansion of genotype G1 (**III**), a large expansion of genotype G3 seemed to have occurred from Iran (**IV**). The Fertile Crescent of the Middle East is considered as one of the earliest centres of livestock domestication (mainly cattle, sheep, pigs and goats) from where the animals were later distributed east- and westwards (Bruford et al., 2003; Zeder, 2008; Chessa et al., 2009; Lv et al., 2015; Rannamäe et al., 2016). These phylogeographic results might therefore reflect the early spread of livestock, infected with G1 and/or G3, from this region. The possible ancestral location of *E. granulosus* s. s. in the Middle East has been suggested before (e.g., Nakao et al., 2010; Casulli et al., 2012; Yanagida et al., 2012; Hassan et al., 2017), but had not been demonstrated using the Bayesian phylogeographic approach.

Although our results point to the Middle East as the origin of G3, it is plausible that a large expansion of this genotype has, in fact, occurred from India, which might not be revealed in the present study due to only a few samples originating from India (n = 4; **IV**). This scenario is also plausible, as it can be speculated that the spread of G3 could be connected to the domestication and subsequent spread of water buffaloes. Two subspecies of the water buffalo, the river and the swamp buffalo, were either both domesticated in the Indian subcontinent (Kierstein et al. 2004) or in the Indus valley region and China, respectively (Kumar et al. 2007; Yindee et al. 2010). Although G3 is no longer regarded as a buffalo-specific genotype and both G1 and G3 seem to be well-adapted to buffaloes (Capuano et al., 2006), the relevance of India in terms of the expansion of G3, is highlighted by the fact that India has the highest global prevalence of genotype G3 (Sharma et al., 2013a). Another clue that the distribution of G3 could be linked to the domestication history of buffaloes, lies in the fact that the high prevalence of G3 coincides with the high prevalence of buffaloes in several regions (Italy, India, Iran and Pakistan) (Capuano et al., 2006; Latif et al., 2010; Sharbatkhori et al., 2011; Sharma et al., 2013a). India is the first country in the world for the number of buffaloes, followed by China and Pakistan (Borghese, 2005). Although the abundance of buffaloes is significantly lower in Europe and the Middle East, the highest numbers of buffaloes in these regions exist in Azerbaijan, Egypt, Italy and Iran (Borghese, 2005). Unfortunately, data on the prevalence of G3 is lacking from several of these countries, which would be highly important to evaluate this correlation. This hypothesis remains to be tested in the future using larger datasets.

For genotype G1, in addition to Turkey, another location from which several diffusion routes originated was Tunisia. Among others, three routes showed a possible migration of genotype G1 from Tunisia to Argentina and Australia (Fig. 7 in **III**). During the 15th and 16th Centuries, sheep and other livestock were introduced to the Americas by Spanish and British colonizers. However, some animals that arrived to the Americas could have had an African origin as some of the livestock species (mostly pigs and goats) were taken aboard on the

Canary Islands, which were colonized by people from North Africa (Rodero et al., 1992; Rando et al., 1999; also discussed in Alvarez Rojas et al., 2017), possibly explaining the significant diffusion route between Tunisia and Argentina. The connection between Tunisia and Australia could also be linked to relatively recent history, as it is thought that the sources of Australian sheep could be Spain and/or North Africa. As discussed in Jenkins (2005), Merinos raised in North Africa arrived in Australia in the beginning of the 19th Century.

Although Argentina assumed the ancestral position to the other American samples (Brazil, Chile and Mexico), this result is counter-intuitive in relation to the direction of livestock introduction to South America (Rodero et al., 1992) and more samples are required from this region to address the parasite's phylogeographic history in this region.

Another interesting result that the analysis revealed was the Algerian origin of the Finnish sample (Fig. 7 in **III**), which was in accordance with the presumed origin of the infection according to the data that we received about the patient. This suggests that implementing high-resolution molecular tools could potentially be used to determine the source of infection in human cases. However, this would require an extensive and high-quality global database of parasite sequences as references, which is currently lacking.

Although the samples in the present study cover most of the global distribution range of genotypes G1 and G3, it is important to note that samples from some geographical regions, in which G1 or G3 have been found to be highly prevalent, were lacking or under-represented (e.g., Peru, Ethiopia, Kenya, Libya and Central Asia for G1; Pakistan and Serbia for G3). In addition, for genotype G1, samples from Argentina, Turkey and Tunisia were in excess compared with other regions. These aspects are highly important to consider in the context of the Bayesian phylogeographic analysis which is highly dependent on sampling and, therefore, should be interpreted with caution. It is also likely that some of the migrations proposed did not occur directly between the two locations, but were in reality much more complex involving geographical locations that were not represented in this study. While we are able to provide the first insight into the large-scale phylogeographic patterns of G1 and G3, these hypotheses should be further tested using larger datasets.

To evaluate whether shorter sequences could also be used to investigate the phylogeographic history of the parasite, we carried out the Bayesian phylogeographic analysis for genotype G3 using the full *cox1* gene (1674 bp) (**IV**). We conducted three independent runs which yielded inconsistent results with low Bayes Factor values. Thus, no significant diffusion routes could be identified based on the *cox1* gene, highlighting that significantly longer sequences are required to investigate the phylogeographic history of the parasite using this approach.

Due to the lack of fossil records to calibrate molecular clocks, the estimation of the divergence time of G1 and G3 remains speculative. One possible explanation to the emergence of the two mitochondrial genotypes could be linked to the Last Glacial Maximum (LGM) (26.5–19 kya), as it has been

widely accepted that climatic fluctuations during this period have shaped the distribution, genetic structure and diversity of present-day species (Hewitt, 2000; Hofreiter and Stewart, 2009; Davison et al., 2011). As continental ice sheets extended into a large part of the temperate zone of the Northern hemisphere, the survival of most organisms was dependent on more hospitable southern refugia (Hewitt, 1999), but also more northern refuge areas, such as the Carpathian Mountains (Kotlík et al., 2006; Saarma et al., 2007; Schmitt and Varga, 2012). For numerous species, the isolation of populations in multiple refugia has resulted in the genetic divergence of mitochondrial lineages, still distinguishable in their mitogenome after post-glacial migrations (e.g., Taberlet and Bouvet, 1994; Santucci et al., 1998; Korsten et al., 2009; McDevitt et al., 2012; Keis et al., 2013; Anijalg et al., 2018). Before *E. granulosus* s. s. became largely adapted to domestic hosts, it most probably circulated in a strictly sylvatic lifecycle and several mitochondrial groups of *E. granulosus* s. s. could have emerged due to separate glacial refugia of the host species. Subsequently, two of these lineages (i.e., ancestors of the present-day G1 and G3) could have given rise to the present *E. granulosus* s. s. populations. Although the present-day mitochondrial lineages of several other species are geographically restricted due to post-glacial migration barriers (e.g., Taberlet and Bouvet, 1994; Hewitt, 1999; Korsten et al., 2009; Davison et al., 2011; Anijalg et al., 2018), obligatory parasites infecting domestic animals have no such barriers due to the transport of host animals between different regions, resulting in the lack of geographic differentiation of the mitochondrial lineages observed for both G1 and G3. While G3 is significantly less prevalent world-wide than G1, it is challenging to propose scenarios that could have led to this contrast. Assuming that G1 and G3 did indeed diverge during the LGM, it is possible that the refugium of G1 could have been significantly larger than that of G3, which could be reflected in the higher global prevalence of G1 even presently. Alternatively, G3 could have been more adapted to fewer host species initially.

4.4. Concluding remarks and prospects for future studies

The main strength of the present thesis lies largely on the high-resolution approach based on near-complete mtDNA sequences and analysis of nuclear loci, which allowed to firmly distinguish the mitochondrial genotypes of *E. granulosus* s. s., confirm the species status of this closely-related cluster and provide deep insight into the patterns of global genetic diversity and phylogeography of this parasite.

The new data that the present thesis provides underpins future research on the distribution, patterns of genetic diversity and evolutionary trajectories of this highly zoonotic species, but also on the potential biological, ecological, genetic or other differences between genotypes G1 and G3. While we were able to provide first insight into the large-scale phylogeographic patterns of G1 and G3,

the hypotheses proposed in the present thesis should be further tested using significantly larger datasets, most importantly covering areas which were underrepresented in the present study (parts of Asia, Africa, South America and Australia). The primers used for the near-complete mtDNA sequencing in the present thesis can be widely applied in deep analysis of the mitogenome of these parasites. However, as the evolutionary history of the mtDNA lineage may differ from that of nuclear DNA, a complete understanding of the historical processes shaping the phylogeographical patterns of *E. granulosus* s. s. could be revealed using the combination of nuclear and mitochondrial data in the future.

Understanding the genetic make-up of this zoonotic species in detail is highly important not only because of the fundamental knowledge that it provides about the parasite, but it is potentially also of practical value. Analyses of the phylogenetic relations of parasite samples provide highly relevant information for the transmission of different genotypes and could thus help to design more effective intervention strategies. As the majority of control programs have been regional (Craig and Larrieu, 2006), attention should shift to global intervention and control programs because of the likely anthropogenic transport of this parasite contributing massively to the worldwide distribution of the parasite, as highlighted in the present thesis. Also, the level of genetic diversity forms the basis for future adaptation of pathogens, for example, potentially constituting a force towards the emergence of new host-parasite associations and for the development of drug resistance (Morgan et al., 2012). Thus, deep analysis of genetic diversity and evolutionary trajectories of various parasites are likely to benefit significantly from large-scale mitochondrial and nuclear genome analyses.

SUMMARY

Cystic echinococcosis (CE) is a zoonotic disease caused by tapeworms within the species complex *Echinococcus granulosus* sensu lato (s. l.). CE is spread worldwide and listed amongst the most severe parasitic diseases in humans, also representing a substantial economic burden on livestock industries. Within this complex, *E. granulosus* sensu stricto (s. s.) is associated with the majority of human CE cases globally and thus merits particular attention.

Within *E. granulosus* s. s., three mitochondrial genotypes (G1–G3) were initially characterised. Although extensive research had been carried out on the genetic structure of this species, significant gaps still existed. As relatively short mitochondrial DNA (mtDNA) sequences had been used so far (up to 1609 bp whereas the full mtDNA of *E. granulosus* s. s. is ~13 500 bp), the full extent of the mitogenome variation within *E. granulosus* s. s. had remained unexplored, hindering detailed analyses of the taxonomy, genetic structure and phylogeographic history of this genotypic group.

The present thesis addressed three key issues that had remained ambiguous thus far. Firstly, one of the most pressing questions was the existence and distinction of *E. granulosus* s. s. mitochondrial genotypes. Analyses of short sequence lengths had demonstrated that G1–G3 are genetically nearly inseparable in phylogenetic networks and the rationale of distinguishing these genotypes in the future had been questioned. However, the distinction and genetic distance of these genotypes based on significantly longer mtDNA sequences, had remained unexplored. Secondly, although a proposal had been made to treat G1–G3 as a single species due to their high genetic similarity based on mtDNA data, the evidence based on nuclear loci was still inconclusive. Thus, despite the assumptions that the mitochondrial genotypes can be regarded as a distinct species, further analysis was required. Thirdly, due to the relatively short sequences used so far, analyses had lacked sufficient phylogenetic power to reveal detailed insight into the patterns of genetic diversity and phylogeography of *E. granulosus* s. s. In addition, due to the ambiguity in the genetic differentiation of G1–G3, no studies so far had attempted to analyse the genotypes separately, revealing possible differences in their phylogeographic history.

The present thesis addressed these issues by sequencing and analysing near-complete mtDNA sequences and several nuclear loci of a large panel of globally distributed *E. granulosus* s. s. samples. Firstly, we demonstrated for the first time that G1 and G3 are genetically clearly distinct genotypes on the basis of near-complete mtDNA data (separated by 37 mutations in the phylogenetic network), whereas our data provided evidence that G2 is not a valid genotype, but belongs to G3. The amount of genetic distinction between G1 and G3 highlighted the importance to use up-to-date molecular techniques to distinguish these genotypes in further analyses. Secondly, we confirmed that G1 and G3 can indeed be regarded as a single species *E. granulosus* s. s., as nuclear data

showed no distinction between the two genotypes, indicating on-going gene flow between them. Thirdly, the analyses of the global patterns of genetic diversity and phylogeography of *E. granulosus* s. s. revealed high genetic variation within genotypes G1 and G3. The high genetic diversity was coupled with low genetic differentiation between G1 and G3 subpopulations globally, particularly across the Mediterranean countries, which is likely the consequence of extensive anthropogenic animal transport and trade. However, slightly lower values of genetic diversity and moderate genetic differentiation was characteristic to South America, possibly due to the more recent arrival of domestic animals to South America compared with the domestication history of livestock in Africa and Eurasia dating back thousands of years BC. The phylogeographic analysis revealed Middle East as the origin of a large-scale expansion of genotypes G1 and G3, a well-known domestication centre for sheep, cattle and goats, which are important intermediate hosts for *E. granulosus* s. s.

The new data that the present thesis presents underpins our fundamental understanding of the genetic make-up of *E. granulosus* s. s. and provides basis for future research on the distribution, patterns of genetic diversity and evolutionary trajectories of this highly zoonotic species.

SUMMARY IN ESTONIAN

Inimesele ohtliku paelussi *Echinococcus granulosus* sensu stricto globaalne geneetiline mitmekesisus ja fülogeograafia

Tsüstiline ehhinokokoos (CE) on zoonootiline haigus, mida põhjustavad *Echinococcus granulosus* sensu lato (s. l.) liigikompleksi kuuluvad paelussid. CE on levinud kõikjal maailmas ning seda peetakse üheks raskemaks parasitaarhaiguseks, mis põhjustab suuri majanduslikke kahjusid. Sellesse kompleksi kuuluvate liikide hulgas on *E. granulosus* sensu stricto (s. s.) eriti oluline olles ülemaailmselt kõige ulatuslikumalt levinud (levik on eriti lai Aafrikas, Austraalias, Lõuna-Euroopas, Lõuna-Ameerikas ja Aasias) ning ka globaalselt kõige sagedasem CE tekitaja inimesel (~88% haigusjuhtudest).

Elutsükli läbimiseks on sellel parasiidiliigil vaja kahte peremeest, nii lõpp- peremeest, kelleks on erinevad koerlased, kui ka vaheperemeest, kelleks on peamiselt sõralised. Lõpp-peremeeste organismis elutseb täiskasvanud uss, vaheperemeeste organismis arenevad tsüstid, milles paiknevad parasiidi vastsed. Tavaliselt ei tekita haigus lõpp-peremeestele märkimisväärseid terviseprobleeme, ent on eluohtlik vaheperemeestele. Liigil *E. granulosus* s. s. on ehhinokokk-paelusside seast kõige laiem peremeesorganismide ring. Sinna hulka kuuluvad lõpp-peremeestest peamiselt koerad, hundid, šaakalid ja dingod ning vaheperemeestest peamiselt lambad, lehmad, kitsed ja pühvlid. Vaheperemeeste hulka kuulub ka inimene, keda peetakse parasiidi tupikperemeheks, kellelt haigus üldjuhul edasi ei kandu. Kui nakkus jääb õigeaegselt ravimata, on see inimesele eluohtlik. Tsüstid võivad areneda erinevates organites, kõige sagedamini maksas või kopsus ning on võimelised saavutama väga suuri mõõtmeid.

Liik *E. granulosus* s. s. arvati koosnevat kolmest genotüübist (G1–G3), mis kirjeldati 1990ndate alguses kahe mitokondriaalse (mtDNA) geenifragmendi põhjal – *cox1*, 366 aluspaari (ap), ning *nad1*, 471 ap. Nendest kahest geenifragmentist said kõige enam kasutud markerid *E. granulosus* s. s. genotüüpide eristamiseks ja geneetilise mitmekesisuse ning struktuuri analüüsimiseks. Harvem kasutati ka pikemaid järjestusi (nt. 1609 ap *cox1* geenist). Kuigi *cox1* ning *nad1* markerid leidsid laialt kasutust ning nende põhjal on tehtud palju olulisi uurimustöid *E. granulosus* s. s. geneetilise mitmekesisuse mõistmiseks eri maailma piirkondades, ilmnes nende kasutamisega aga üha enam probleeme. G1–G3 genotüüpide sisene geneetiline variatsioon oli osutunud mitmeid kordi kõrgemaks kui algselt kirjeldatud, mille tõttu ei olnud võimalik nende markerite põhjal suurt hulka parasiidiproove genotüübi täpsuseni määrata. Samuti leiti, et genotüüpide vaheline geneetiline erinevus on väga väike (1–2 mutatsiooni) ning *E. granulosus* s. s. siseste selgelt eristuvate genotüübigruppide olemasolu pandi kahtluse alla. Samas, kuna siiani oli analüüsides kasutatud lühikesi mitokondriaalseid järjestusi, oli kogu mtDNA (~13 500 ap) geneetilise variatsiooni ulatus teadmata ning sellest lähtuvalt esines mitmeid olulisi lünki.

Käesolev doktoritöö keskendus peamiselt kolmele lahendamata küsimusele. Esimeseks oluliseks probleemiks oli *E. granulosus* s. s. mitokondriaalsete geno-

tüüpide olemasolu tuvastamine ning üksteisest eristamine. Seega oli doktoritöö üheks eesmärgiks teha kindlaks mitmest genotüübist *E. granulosus* s. s. koosneb ning kui suur on nende vaheline geneetiline distants. Selleks sekveneeriti suurel hulgal parasiidiproovidel mitokondri genoomi pea täies ulatuses. Teiseks, kuigi oli välja pakutud, et G1–G3 on mitogenoomi geneetilise sarnasuse alusel üks liik, ei olnud seda teaduslikult näidatud. Selleks oli vaja täiendavaid tuumageenide analüüse. Doktoritöö käigus sekveneeriti esmakordselt kõigil *E. granulosus* s. s. genotüüpidel kolm tuumalookust. Kolmandaks, kuna senised geneetilise mitmekesisuse ning fülogeograafia analüüsid põhinesid lühikestel mtDNA järjestusel, ei võimaldanud need mõista detailseid mustreid vähese fülogeneetilise eristusvõime tõttu. Lisaks olid senised tööd olnud lokaalsed, mistõttu puudus globaalne ülevaade. Kuna siiani oli paljusid G1–G3 proove geneetiliselt võimatu genotüübi täpsuseni määrata, siis ei olnud G1–G3 eraldi analüüsitud. See võimaldaks aga tuvastada erinevusi nende geneetilise mitmekesisuse mustrites. Selleks analüüsisime mitogenoomi pea täisjärjestuse alusel eri genotüüpide geneetilist struktuuri ning fülogeograafilisi mustreid.

Kokku analüüsiti käesolevas doktoritöö käigus 293 *E. granulosus* s. s. proovi nii Lõuna-Ameerikast, Aafrikast, Euroopast, Aasiast kui ka Austraaliast. Proovid pärinesid erinevatelt peremeesliikidelt: lammas, veis, inimene, metssiga, kodusiga, kits, pühvel, kaamel ning dingo. Samuti kaasati analüüsi üks *E. equinus* (genotüüp G4) proov eeslilt ning 3 *E. ortleppi* (genotüüp G5) proovi pühvlilt. Töötasime välja 27 uut praimerit mitokondri genoomi pea täies pikkuses sekveneerimiseks (kuni 11 678 ap). Kolme juba varem publitseeritud praimeripaariga sekveneeriti ka kolm tuumalookust (kokku 2984 ap). DNA järjestused assambleeriti ning kontrolliti kasutades programme CodonCode, BioEdit ning Geneious. Järjestustega viidi läbi nii fülogeneetilisi kui ka –geograafilisi analüüse kasutades peamiselt programme Network ning BEAUti & The BEAST. Programmidega DnaSP ning Arlequin arvutati erinevad populatsiooni indeksid (haplotüüpide mitmekesisus, nukleotiidide mitmekesisus, neutraalsus indeksid Tajima's D ja Fs ning populatsioonide paaridevaheline Fst), mis väljendavad ning võrdlevad populatsioonide sisest ning vahelist geneetilist mitmekesisust ning struktuuri.

Olulisemad tulemused antud doktoritöös on järgmised. Esiteks tegime kindlaks, et *E. granulosus* s. s. koosneb kahest selgelt eristuvast mitokondriaalsest genotüübist G1 ja G3 ning näitasime, et G2 ei ole eraldi genotüüp, vaid kuulub G3 hulka. Analüüsitud 293-st *E. granulosus* s. s. proovist 254 kuulus genotüüpi G1 ja 39 genotüüpi G3. Fülogeneetilisel võrgustikul eristas neid genotüüpe 37 mutatsiooni, mis näitab, et tegemist on selgelt eristuvate mitokondriaalsete gruppidega. Neli proovi osutusid algse molekulaarse definitsiooni alusel genotüübiks G2. Need aga klasterdusid kokku G3 proovidega ning ei olnud fülogeneetilisel võrgustikul monofüleetilised. Seetõttu on alust järeldada, et G2 ei ole eraldi genotüüp, vaid kuulub G3 hulka. Teiseks tegime kindlaks, et G1 ning G3 võib tõepoolest lugeda ühte liiki (*E. granulosus* s. s.) kuuluvateks mitokondriaalseteks genotüüpideks, kuna tuumalookuste analüüsil ei eristunud need genotüübid teineteisest, viidates nende vahelisele geenivoolule. Kolman-

daks iseloomustasime G1 ning G3 globaalset geneetilist mitmekesisust, struktuuri ning fülogeograafiat. Näitasime, et mõlema genotüübi geneetiline mitmekesisus on globaalselt väga kõrge, kuigi G3 arvukus on ülemaailmselt oluliselt väiksem ja levik piiratum. Samuti oli mõlemale genotüübile iseloomulik erinevate alampopulatsioonide vähene geneetiline diferentseerumine. Kuna *E. granulosus* s. s. nakatab peamiselt koduloomi, on praegust parasiidi levikut suure tõenäosusega mõjutanud intensiivne loomakaubandus ning -transport, mis on suuresti lihtsustanud parasiidi kiiret levikut eri piirkondade ning ka geograafiliselt väga kaugete riikide vahel. See väljendub erinevatest piirkondadest kogutud parasiidiproovide geneetilises sarnasuses. Samas oli Lõuna-Ameerikas geneetiline mitmekesisus mõnevõrra madalam ning geneetiline diferentseerumine teistest alampopulatsioonidest kõrgem. On võimalik, et seda on põhjustanud koduloomade hilisem jõudmine Lõuna-Ameerikasse võrreldes nende loomade kodustamise pika ajalooga Aafrikas ning Euraasias. Fülogeograafilise analüüsi tulemused näitasid, et genotüüpide G1 ning G3 üheks suuremaks ekspansiooni keskpunktiks on olnud Lähis-Ida, mis on hästi tuntud ka lamba, veise ja kitse kodustamise piirkonnana – kõik need liigid on olulised *E. granulosus* s. s. vaheperemehed. Seega on võimalik, et sellesse haigusesse nakatunud koduloomi leidus juba kodustamise algusest saati ning haigus levis edasi teistesse piirkondadesse kariloomade kasvatuse edasise levimise tõttu. Fülogeograafilise analüüsi huvitavaks tulemuseks oli ka Soome inimese proovi Alžeeria päritolu. Kuna selle proovi kohta oli eelnevalt teada, et nakkus võib olla saadud Alžeeriast, näitab see, et kasutades suure lahutusvõimega pikki mtDNA järjestusi, on Bayesi statistikale põhineva metoodika abil võimalik määrata inimnakkuste võimalikku päritolu.

Käesolev doktoritöö kirjeldas esmakordselt inimtervise seisukohalt äärmiselt olulise parasiidiliigi *E. granulosus* s. s. geneetilist varieeruvust ning globaalset struktuuri ning tõi välja pikkade mitokondriaalsete järjestuste kasutamisel saavutatava kõrgema lahutusvõimega analüüside selged eelised, mis võimaldasid täita olulisi lünki senistes teadmistes. Töö on vundamendiks tulevastele analüüsidele selle liigi genotüüpide leviku, geneetilise mitmekesisuse ning fülogeograafia vallas.

REFERENCES

Abushhewa, M.H., Abushhiwa, M.H.S., Nolan, M.J., Jex, A.R., Campbell, B.E., Jabbar, A., Gasser, R.B., 2010. Genetic classification of *Echinococcus granulosus* cysts from humans, cattle and camels in Libya using mutation scanning-based analysis of mitochondrial loci. Mol. Cell. Probes 24, 346–351. https://doi.org/10.1016/j.mcp.2010.07.005

Addy, F., Alakonya, A., Wamae, N., Magambo, J., Mbae, C., Mulinge, E., Zeyhle, E., Wassermann, M., Kern, P., Romig, T., 2012. Prevalence and diversity of cystic echinococcosis in livestock in Maasailand, Kenya. Parasitol. Res. 111, 2289–2294. https://doi.org/10.1007/s00436-012-3082-8

Alvarez Rojas, C.A., Ebi, D., Gauci, C.G., Scheerlinck, J.P., Wassermann, M., Jenkins, D.J., Lightowlers, M.W., Romig, T., 2016. Microdiversity of *Echinococcus granulosus* sensu stricto in Australia. Parasitology 143, 1026–1033. doi:10.1017/S0031182016000445

Alvarez Rojas, C.A., Ebi, D., Paredes, R., Acosta-Jamett, G., Urriola, N., Roa, J.C., Manterola, C., Cortes, S., Romig, T., Scheerlinck, J.-P., Lightowlers, M.W., 2017. High intraspecific variability of *Echinococcus granulosus* sensu stricto in Chile. Parasitol. Int. 66, 112–115. doi:10.1016/j.parint.2016.12.001

Alvarez Rojas, C.A., Romig, T., Lightowlers, M.W., 2014. *Echinococcus granulosus* sensu lato genotypes infecting humans – review of current knowledge. Int. J. Parasitol. 44, 9–18. doi:10.1016/j.ijpara.2013.08.008

Andresiuk, M.V., Gordo, F.P., Saarma, M., Elissondo, M.C., Taraborelli, A., Casalongue, C., Denegri, G., Saarma, U., 2013. *Echinococcus granulosus* genotype G1 dominated in cattle and sheep during 2003–2006 in Buenos Aires province, an endemic area for cystic echinococcosis in Argentina. Acta Trop. 127, 136–142. doi:10.1016/j.actatropica.2013.04.008

Anijalg, P., Ho, S.Y.W., Davison, J., Keis, M., Tammeleht, E., Bobowik, K., Tumanov, I.L., Saveljev, A.P., Lyapunova, E.A., Vorobiev, A.A., Markov, N.I., Kryukov, A.P., Kojola, I., Swenson, J.E., Hagen, S.B., Eiken, H.G., Paule, L., Saarma, U., 2018. Large-scale migrations of brown bears in Eurasia and to North America during the Late Pleistocene. J. Biogeogr. 45, 394–405. https://doi.org/10.1111/jbi.13126

Ayres, D.L., Darling, A., Zwickl, D.J., Beerli, P., Holder, M.T., Lewis, P.O., Huelsenbeck, J.P., Ronquist, F., Swofford, D.L., Cummings, M.P., Rambaut, A., Suchard, M.A., 2012. BEAGLE: an application programming interface and high-performance computing library for statistical phylogenetics. Syst. Biol. 61, 170–173. doi:10.1093/sysbio/syr100

Bandelt, H.J., Forster, P., Rohl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48.

Bart, J.M., Morariu, S., Knapp, J., Ilie, M.S., Pitulescu, M., Anghel, A., Cosoroaba, I., Piarroux, R., 2006. Genetic typing of *Echinococcus granulosus* in Romania. Parasitol. Res. 98, 130–137. https://doi.org/10.1007/s00436-005-0015-9

Bielejec, F., Baele, G., Vrancken, B., Suchard, M.A., Rambaut, A., Lemey, P., 2016. Spread3: Interactive visualization of spatiotemporal history and trait evolutionary processes. Mol. Biol. Evol. 33, 2167–2169. doi:10.1093/molbev/msw082

Borghese, A., 2005. Buffalo production and research, Food and Agriculture Organization of the United Nations, Rome.

Boufana, B., Lahmar, S., Rebaï, W., Safta, Z.B., Jebabli, L., Ammar, A., Kachti, M., Aouadi, S., Craig, P.S., 2014. Genetic variability and haplotypes of *Echinococcus* isolates from Tunisia. Trans. R. Soc. Trop. Med. Hyg. 108, 706–714. doi:10.1093/trstmh/tru138

Boufana, B., Lett, W.S., Lahmar, S., Buishi, I., Bodell, A.J., Varcasia, A., Casulli, A., Beeching, N.J., Campbell, F., Terlizzo, M., McManus, D.P., Craig, P.S., 2015. *Echinococcus equinus* and *Echinococcus granulosus* sensu stricto from the United Kingdom: genetic diversity and haplotypic variation. Int. J. Parasitol. 45, 161–166. doi:10.1016/j.ijpara.2014.10.005

Bowles, J., Blair, D., McManus, D.P., 1992. Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. Mol. Biochem. Parasitol. 54, 165–173.

Bowles, J., Blair, D., McManus, D., 1994. Molecular genetic characterization of the cervid strain ('northern form') of *Echinococcus granulosus*. Parasitology 109, 215–221.

Bowles, J., McManus, D., 1993. Nadh dehydrogenase-1 gene-sequences compared for species and strains of the genus *Echinococcus*. Int. J. Parasitol. 23, 969–972. https://doi.org/10.1016/0020-7519(93)90065-7

Breyer, I., Georgieva, D., Kurdova, R., Gottstein, B., 2004. *Echinococcus granulosus* strain typing in Bulgaria: the G1 genotype is predominant in intermediate and definitive wild hosts. Parasitol. Res. 93, 127–130. https://doi.org/10.1007/s00436-004-1116-6

Bruford, M.W., Bradley, D.G., Luikart, G., 2003. DNA markers reveal the complexity of livestock domestication. Nat. Rev. Genet. 4, 900–910. doi:10.1038/nrg1203

Brunetti, E., Kern, P., Vuitton, D.A., Writing Panel for the WHO-IWGE, 2010. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Trop. 114, 1–16. https://doi.org/10.1016/j.actatropica.2009.11.001

Budke, C.M., Casulli, A., Kern, P., Vuitton, D.A., 2017. Cystic and alveolar echinococcosis: Successes and continuing challenges. PLoS Negl. Trop. Dis. 11, e0005477. doi:10.1371/journal.pntd.0005477

Busi, M., Snábel, V., Varcasia, A., Garippa, G., Perrone, V., De Liberato, C., D'Amelio, S., 2007. Genetic variation within and between G1 and G3 genotypes of *Echinococcus granulosus* in Italy revealed by multilocus DNA sequencing. Vet. Parasitol. 150, 75–83. https://doi.org/10.1016/j.vetpar.2007.09.003

Capuano, F., Rinaldi, L., Maurelli, M.P., Perugini, A.G., Veneziano, V., Garippa, G., Genchi, C., Musella, V., Cringoli, G., 2006. Cystic echinococcosis in water buffaloes: epidemiological survey and molecular evidence of ovine (G1) and buffalo (G3) strains. Vet. Parasitol. 137, 262–268. https://doi.org/10.1016/j.vetpar.2006.01.016

Cardona, G.A., Carmena, D., 2013. A review of the global prevalence, molecular epidemiology and economics of cystic echinococcosis in production animals. Vet. Parasitol. 192, 10–32. https://doi.org/10.1016/j.vetpar.2012.09.027

Casulli, A., Interisano, M., Sreter, T., Chitimia, L., Kirkova, Z., La Rosa, G., Pozio, E., 2012. Genetic variability of *Echinococcus granulosus* sensu stricto in Europe inferred by mitochondrial DNA sequences. Infect. Genet. Evol. 12, 377–383. doi:10.1016/j.meegid.2011.12.014

Chessa, B., Pereira, F., Arnaud, F., Amorim, A., Goyache, F., Mainland, I., Kao, R., Pemberton, J., Beraldi, D., Stear, M., Alberti, A., Pittau, M., Iannuzzi, L., Banabazi, M., Kazwala, R., Zhang, Y., Arranz, J.J., Ali, B., Wang, Z., Uzun, M., Dione, M., Olsaker, I., Holm, L.-E., Saarma, U., Ahmad, S., Marzanov, N., Eythorsdottir, E.,

Holland, M., Ajmone-Marsan, P., Bruford, M., Kantanen, J., Spencer, T., Palmarini, M., 2009. Revealing the history of sheep domestication using retrovirus integrations. Science 324, 532–536. doi:10.1126/science.1170587

Craig, P.S., Larrieu, E., 2006. Control of cystic echinococcosis/hydatidosis, 1863–2002. Adv. Parasitol. 61, 443–508.

Cucher, M.A., Macchiaroli, N., Baldi, G., Camicia, F., Prada, L., Maldonado, L., Avila, H.G., Fox, A., Gutiérrez, A., Negro, P., Lopez, R., Jensen, O., Rosenzvit, M., Kamenetzky, L., 2016. Cystic echinococcosis in South America: systematic review of species and genotypes of *Echinococcus granulosus* sensu lato in humans and natural domestic hosts. Trop. Med. Int. Health 21, 166–175. doi:10.1111/tmi.12647

Dakkak, A., 2010. Echinococcosis/hydatidosis: a severe threat in Mediterranean countries. Vet. Parasitol. 174, 2–11. doi: 10.1016/j.vetpar.2010.08.009

Davison, J., Ho, S.Y.W., Bray, S.C., Korsten, M., Tammeleht, E., Hindrikson, M., Østbye, K., Østbye, E., Lauritzen, S.-E., Austin, J., Cooper, A., Saarma, U., 2011. Late-Quaternary biogeographic scenarios for the brown bear (*Ursus arctos*), a wild mammal model species. Quat. Sci. Rev. 30, 418–430. https://doi.org/10.1016/j.quascirev.2010.11.023

Debeljak, Z., Boufana, B., Interisano, M., Vidanovic, D., Kulisic, Z., Casulli, A., 2016. First insights into the genetic diversity of *Echinococcus granulosus* sensu stricto (s.s.) in Serbia. Vet. Parasitol. 223, 57–62. https://doi.org/10.1016/j.vetpar.2016.04.007

de la Rue, M.L., Takano, K., Brochado, J.F., Costa, C.V., Soares, A.G., Yamano, K., Yagi, K., Katoh, Y., Takahashi, K., 2011. Infection of humans and animals with *Echinococcus granulosus* (G1 and G3 strains) and *E. ortleppi* in Southern Brazil. Vet. Parasitol. 177, 97–103. https://doi.org/10.1016/j.vetpar.2010.11.018

Deplazes, P., Rinaldi, L., Alvarez Rojas, C.A., Torgerson, P.R., Harandi, M.F., Romig, T., Antolova, D., Schurer, J.M., Lahmar, S., Cringoli, G., Magambo, J., Thompson, R.C.A., Jenkins, E.J., 2017. Global distribution of alveolar and cystic echinococcosis. Adv. Parasitol. 95, 315–493. https://doi.org/10.1016/bs.apar.2016.11.001

Deplazes, P., van Knapen, F., Schweiger, A., Overgaauw, P.A.M., 2011. Role of pet dogs and cats in the transmission of helminthic zoonoses in Europe, with a focus on echinococcosis and toxocarosis. Vet. Parasitol. 182, 41–53. https://doi.org/10.1016/j.vetpar.2011.07.014

Drummond, A.J., Bouckaert, R.R., 2015. Bayesian evolutionary analysis with BEAST. Cambridge University Press, Cambridge, UK.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973. doi:10.1093/molbev/mss075

Eckert, J., Deplazes, P., 2004. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin. Microbiol. Rev. 17, 107–135. https://doi.org/10.1128/CMR.17.1.107-135.2004

Eckert, J., Deplazes, P., Craig, P., Gemmell, M., Gottstein, B., Heath, D., Jenkins, D., Kamiya, M., Lightowlers, M., Meslin, F., 2001. Echinococcosis in animals: clinical aspects, diagnosis and treatment. WHO/OIE Manual on echinococcosis in humans and animals: a public health problem of global concern.

Eckert, J., Thompson, R.C.A., 2017. Historical aspects of echinococcosis. Adv. Parasitol. 95, 1–64. https://doi.org/10.1016/bs.apar.2016.07.003

Ehsan, M., Akhter, N., Bhutto, B., Arijo, A., Ali Gadahi, J., 2017. Prevalence and genotypic characterization of bovine *Echinococcus granulosus* isolates by using

cytochrome oxidase 1 (Co1) gene in Hyderabad, Pakistan. Vet. Parasitol. 239, 80–85. https://doi.org/10.1016/j.vetpar.2017.04.006

Espinoza, S., Salas, A.M., Vargas, A., Freire, V., Diaz, E., Sánchez, G., Venegas, J., 2014. Detection of the G3 genotype of *Echinococcus granulosus* from hydatid cysts of Chilean cattle using COX1 and ND1 mitochondrial markers. Parasitol. Res. 113, 139–147. https://doi.org/10.1007/s00436-013-3636-4

Excoffier, L., Laval, G. and Schneider, S., 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol. Bioinform. Online 1, 47–50.

Food and Agriculture Organization of the United Nations/World Health Organization, 2014. Multicriteria-based ranking for risk management of food-borne parasites. Microbiological Risk Assessment Series, Rome.

Faria, N.R., Suchard, M.A., Rambaut, A., Lemey, P., 2011. Toward a quantitative understanding of viral phylogeography. Curr. Opin. Virol. 1, 423–429. doi:10.1016/j.coviro.2011.10.003

Fu, Y.X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147, 915–925.

Griffiths, R.C., Tavaré, S., 1994. Sampling theory for neutral alleles in a varying environment. Philos. Trans. R. Soc. Lond. B Biol. Sci. 344, 403–410.

Gu, X., Fu, Y.-X., Li, W.-H., 1995. Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. Mol. Biol. Evol. 12, 546–557.

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321. doi:10.1093/sysbio/syq010

Guo, Z.H., Kubo, M., Kudo, M., Nibe, K., Horii, Y., Nonaka, N., 2011. Growth and genotypes of *Echinococcus granulosus* found in cattle imported from Australia and fattened in Japan. Parasitol. Int. 60, 498–502. https://doi.org/10.1016/j.parint.2011.09.002

Hajialilo, M., Fasihi Harandi, M., Sharbatkhori, M., Mirhendi, H., Rostami, S., 2012. Genetic characterization of *Echinococcus granulosus* in camels, cattle and sheep from the south-east of Iran indicates the presence of the G3 genotype. J. Helminthol. 86, 263–270. doi:10.1017/S0022149X11000320

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hassan, Z.I., Meerkhan, A.A., Boufana, B., Hama, A.A., Ahmed, B.D., Mero, W.M.S., Orsten, S., Interisano, M., Pozio, E., Casulli, A., 2017. Two haplotype clusters of *Echinococcus granulosus* sensu stricto in northern Iraq (Kurdistan region) support the hypothesis of a parasite cradle in the Middle East. Acta Trop. 172, 201–207. doi:10.1016/j.actatropica.2017.04.028

Hewitt, G.M., 1999. Post□glacial re□colonization of European biota. Biol. J. Linn. Soc. 68, 87–112. https://doi.org/10.1111/j.1095-8312.1999.tb01160.x

Hewitt, G., 2000. The genetic legacy of the Quaternary ice ages. Nature 405, 907–913. https://doi.org/10.1038/35016000

Hofreiter, M., Stewart, J., 2009. Ecological change, range fluctuations and population dynamics during the Pleistocene. Curr. Biol. CB 19, R584–594. https://doi.org/10.1016/j.cub.2009.06.030

Hotez, P.J., Brindley, P., Bethony, J.M., King, C.H., Pearce, E.J., Jacobson, J., 2008. Helminth infections: the great neglected tropical diseases. J. Clin. Investig. 118, 1311–1321.

Hüttner, M., Nakao, M., Wassermann, T., Siefert, L., Boomker, J.D.F., Dinkel, A., Sako, Y., Mackenstedt, U., Romig, T., Ito, A., 2008. Genetic characterization and phylogenetic position of *Echinococcus felidis* Ortlepp, 1937 (Cestoda: Taeniidae) from the African lion. Int. J. Parasitol. 38, 861–868. doi:10.1016/j.ijpara.2007.10.013

Jabbar, A., Narankhajid, M., Nolan, M.J., Jex, A.R., Campbell, B.E., Gasser, R.B., 2011. A first insight into the genotypes of *Echinococcus granulosus* from humans in Mongolia. Mol. Cell. Probes 25, 49–54. https://doi.org/10.1016/j.mcp.2010.11.001

Jenkins, D.J., 2005. Hydatid control in Australia: where it began, what we have achieved and where to from here. Int. J. Parasitol. 35, 733–740.

Jenkins, D.J., Romig, T., Thompson, R.C.A., 2005. Emergence/re-emergence of *Echinococcus* spp. – a global update. Int. J. Parasitol. 35, 1205–1219. https://doi.org/10.1016/j.ijpara.2005.07.014

Kamenetzky, L., Gutierrez, A.M., Canova, S.G., Haag, K.L., Guarnera, E.A., Parra, A., García, G.E., Rosenzvit, M.C., 2002. Several strains of *Echinococcus granulosus* infect livestock and humans in Argentina. Infect. Genet. Evol. 2, 129–136.

Keis, M., Remm, J., Ho, S.Y.W., Davison, J., Tammeleht, E., Tumanov, I.L., Saveljev, A.P., Männil, P., Kojola, I., Abramov, A.V., Margus, T., Saarma, U., 2013. Complete mitochondrial genomes and a novel spatial genetic method reveal cryptic phylogeographical structure and migration patterns among brown bears in north-western Eurasia. J. Biogeogr. 40, 915–927. https://doi.org/10.1111/jbi.12043

Kern, P., Menezes da Silva, A., Akhan, O., Müllhaupt, B., Vizcaychipi, K.A., Budke, C., Vuitton, D.A., 2017. The echinococcoses: diagnosis, clinical management and burden of disease. Adv. Parasitol. 96, 259–369. https://doi.org/10.1016/bs.apar.2016.09.006

Kierstein, G., Vallinoto, M., Silva, A., Schneider, M.P., Iannuzzi, L., Brenig, B., 2004. Analysis of mitochondrial D-loop region casts new light on domestic water buffalo (*Bubalus bubalis*) phylogeny. Mol. Phylogenet. Evol. 30, 308–324.

Kinkar, L., Laurimäe, T., Acosta-Jamett, G., Andresiuk, V., Balkaya, I., Casulli, A., Gasser, R.B., van der Giessen, J., González, L.M., Haag, K.L., Zait, H., Irshadullah, M., Jabbar, A., Jenkins, D.J., Kia, E.B., Manfredi, M.T., Mirhendi, H., M'rad, S., Rostami-Nejad, M., Oudni-M'rad, M., Pierangeli, N.B., Ponce-Gordo, F., Rehbein, S., Sharbatkhori, M., Simsek, S., Soriano, S.V., Sprong, H., Šnábel, V., Umhang, G., Varcasia, A., Saarma, U., 2018a. Global phylogeography and genetic diversity of the zoonotic tapeworm *Echinococcus granulosus* sensu stricto genotype G1. Int. J. Parasitol. doi: 10.1016/j.ijpara.2018.03.006

Kinkar, L., Laurimäe, T., Balkaya, I., Casulli, A., Zait, H., Irshadullah, M., Sharbatkhori, M., Mirhendi, H., Rostami-Nejad, M., Ponce-Gordo, F., Rehbein, S., Kia, E.B., Simsek, S., Šnábel, V., Umhang, G., Varcasia, A., Saarma, U., 2018b. Genetic diversity and phylogeography of the elusive, but epidemiologically important *Echinococcus granulosus* sensu stricto genotype G3. Parasitology. doi: 10.1017/S0031182018000549

Kinkar, L., Laurimäe, T., Acosta-Jamett, G., Andresiuk, V., Balkaya, I., Casulli, A., Gasser, R.B., González, L.M., Haag, K.L., Zait, H., Irshadullah, M., Jabbar, A., Jenkins, D.J., Manfredi, M.T., Mirhendi, H., M'rad, S., Rostami-Nejad, M., Oudni-M'rad, M., Pierangeli, N.B., Ponce-Gordo, F., Rehbein, S., Sharbatkhori, M., Kia,

E.B., Simsek, S., Soriano, S.V., Sprong, H., Šnábel, V., Umhang, G., Varcasia, A., Saarma, U., 2018c. Distinguishing *Echinococcus granulosus* sensu stricto genotypes G1 and G3 with confidence: a practical guide. Infect. Genet. Evol.

Kinkar, L., Laurimäe, T., Sharbatkhori, M., Mirhendi, H., Kia, E.B., Ponce-Gordo, F., Andresiuk, V., Simsek, S., Lavikainen, A., Irshadullah, M., Umhang, G., Oudni-M'rad, M., Acosta-Jamett, G., Rehbein, S., Saarma, U., 2017. New mitogenome and nuclear evidence on the phylogeny and taxonomy of the highly zoonotic tapeworm *Echinococcus granulosus* sensu stricto. Infect. Genet. Evol. 52, 52–58. doi: 10.1016/j.meegid.2017.04.023

Kinkar, L., Laurimäe, T., Simsek, S., Balkaya, I., Casulli, A., Manfredi, M.T., Ponce-Gordo, F., Varcasia, A., Lavikainen, A., González, L.M., Rehbein, S., van der Giessen, J., Sprong, H., Saarma, U., 2016. High-resolution phylogeography of zoonotic tapeworm *Echinococcus granulosus* sensu stricto genotype G1 with an emphasis on its distribution in Turkey, Italy and Spain. Parasitology 143, 1790. doi: 10.1017/S0031182016001530

Knapp, J., Gottstein, B., Saarma, U., Millon, L., 2015. Taxonomy, phylogeny and molecular epidemiology of *Echinococcus multilocularis*: from fundamental knowledge to health ecology. Vet. Parasitol. 213, 85–91. doi:10.1016/j.vetpar.2015.07.030

Knapp, J., Nakao, M., Yanagida, T., Okamoto, M., Saarma, U., Lavikainen, A., Ito, A., 2011. Phylogenetic relationships within *Echinococcus* and *Taenia* tapeworms (Cestoda: Taeniidae): an inference from nuclear protein-coding genes. Mol. Phylogen. Evol. 61, 628–638. doi:10.1016/j.ympev.2011.07.022

Korsten, M., Ho, S.Y.W., Davison, J., Pähn, B., Vulla, E., Roht, M., Tumanov, I.L., Kojola, I., Andersone-Lilley, Z., Ozolins, J., Pilot, M., Mertzanis, Y., Giannakopoulos, A., Vorobiev, A.A., Markov, N.I., Saveljev, A.P., Lyapunova, E.A., Abramov, A.V., Männil, P., Valdmann, H., Pazetnov, S.V., Pazetnov, V.S., Rõkov, A.M., Saarma, U., 2009. Sudden expansion of a single brown bear maternal lineage across northern continental Eurasia after the last ice age: a general demographic model for mammals? Mol. Ecol. 18, 1963–1979.

Kotlík, P., Deffontaine, V., Mascheretti, S., Zima, J., Michaux, J.R., Searle, J.B., 2006. A northern glacial refugium for bank voles (*Clethrionomys glareolus*). Proc. Natl. Acad. Sci. 103, 14860–14864. https://doi.org/10.1073/pnas.0603237103

Kumar, S., Nagarajan, M., Sandhu, J.S., Kumar, N., Behl, V., Nishanth, G., 2007. Mitochondrial DNA analyses of Indian water buffalo support a distinct genetic origin of river and swamp buffalo. Anim. Genet. 38, 227–232. https://doi.org/10.1111/j.1365-2052.2007.01602.x

Lanfear, R., Calcott, B., Ho, S.Y., Guindon, S., 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29, 1695–1701. doi:10.1093/molbev/mss020

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., Calcott, B., 2016. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. 34, 772–773. doi:10.1093/molbev/msw260

Latif, A.A., Tanveer, A., Maqbool, A., Siddiqi, N., Kyaw-Tanner, M., Traub, R.J., 2010. Morphological and molecular characterisation of *Echinococcus granulosus* in livestock and humans in Punjab, Pakistan. Vet. Parasitol. 170, 44–49. https://doi.org/10.1016/j.vetpar.2010.02.003

Laurimäe, T., Kinkar, L., Andresiuk, V., Haag, K.L., Ponce-Gordo, F., Acosta-Jamett, G., Garate, T., González, L.M., Saarma, U., 2016. Genetic diversity and phylogeo-

graphy of highly zoonotic *Echinococcus granulosus* genotype G1 in the Americas (Argentina, Brazil, Chile and Mexico) based on 8279bp of mtDNA. Infect. Genet. Evol. 45, 290–296. doi:10.1016/j.meegid.2016.09.015

Laurimäe, T., Kinkar, L., Moks, E., Romig, T., Omer, R.A., Casulli, A., Umhang, G., Bagrade, G., Irshadullah, M., Sharbatkhori, M., Mirhendi, H., Ponce-Gordo, F., Soriano, S.V., Varcasia, A., Rostami-Nejad, M., Andresiuk, V., Saarma, U., 2018a. Molecular phylogeny based on six nuclear genes suggests that *Echinococcus granulosus* sensu lato genotypes G6/G7 and G8/G10 can be regarded as two distinct species. Parasitology. https://doi.org/10.1017/S0031182018000719

Laurimäe, T., Kinkar, L., Romig, T., Omer, R.A., Casulli, A., Umhang, G., Gasser, R., Jabbar, A., Sharbatkori, M., Mirhendi, H., Ponce-Gordo, F., Lazzarini, L., Soriano, S.V., Varcasia, A., Rostami-Nejad, M., Andresiuk, V., Maravilla, P., González, L., Dybicz, M., Gawor, J., Šarkunas, M., Snabel, V., Kuzmina, T., Saarma, U., 2018b. The benefits of analysing complete mitochondrial genomes: deep insights into the phylogeny and population structure of *Echinococcus granulosus* sensu lato genotypes G6 and G7. Infect. Genet. Evol. https://doi.org/10.1016/j.meegid.2018.06.016

Lavikainen, A., Lehtinen, M., Meri, T., Hirvelä-Koski, V., Meri, S., 2003. Molecular genetic characterization of the Fennoscandian cervid strain, a new genotypic group (G10) of *Echinococcus granulosus*. Parasitology 127, 207–215.

Lemey, P., Rambaut, A., Drummond, A.J., Suchard, M.A., 2009. Bayesian phylogeography finds its roots. PLoS Comput Biol 5, e1000520. doi:10.1371/journal.pcbi.1000520

Librado, P., Rozas, J., 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452. doi:10.1093/bioinformatics/btp187

Lindquist, H.D.A., Cross, J.H., 2017. 195 – Helminths, in: Cohen, J., Powderly, W.G., Opal, S.M. (Eds.), Infectious Diseases (Fourth Edition). Elsevier, 1763–1779. https://doi.org/10.1016/B978-0-7020-6285-8.00195-7

Lv, F.-H., Peng, W.-F., Yang, J., Zhao, Y.-X., Li, W.-R., Liu, M.-J., Ma, Y.-H., Zhao, Q.-J., Yang, G.-L., Wang, F., Li, J.-Q., Liu, Y.-G., Shen, Z.-Q., Zhao, S.-G., Hehua, E., Gorkhali, N.A., Farhad Vahidi, S.M., Muladno, M., Naqvi, A.N., Tabell, J., Iso-Touru, T., Bruford, M.W., Kantanen, J., Han, J.-L., Li, M.-H., 2015. Mitogenomic meta-analysis identifies two phases of migration in the history of eastern Eurasian sheep. Mol. Biol. Evol. 32, 2515–2533. doi:10.1093/molbev/msv139

Lymbery, A.J., 2017. Phylogenetic pattern, evolutionary processes and species delimitation in the genus *Echinococcus*. Adv. Parasitol. 95, 111–145. https://doi.org/10.1016/bs.apar.2016.07.002

Lymbery, A.J., Jenkins, E.J., Schurer, J.M., Thompson, R.C.A., 2015. *Echinococcus canadensis*, *E. borealis*, and *E. intermedius*. What's in a name? Trends Parasitol. 31, 23–29.

McDevitt A.D., Zub, K., Kawałko, A., Oliver, M.K., Herman J.S., Wójcik J.M., 2012. Climate and refugial origin influence the mitochondrial lineage distribution of weasels (*Mustela nivalis*) in a phylogeographic suture zone. Biol. J. Linn. Soc. 106, 57–69. https://doi.org/10.1111/j.1095-8312.2012.01840.x

Mitrea, I.L., Ionita, M., Costin, I.I., Predoi, G., Avram, E., Rinaldi, L., Maurelli, M.P., Cringoli, G., Genchi, C., 2014. Occurrence and genetic characterization of *Echi-*

nococcus granulosus in naturally infected adult sheep and cattle in Romania. Vet. Parasitol. 206, 159–166. https://doi.org/10.1016/j.vetpar.2014.10.028

Moks, E., Jõgisalu, I., Valdmann, H., Saarma, U., 2008. First report of *Echinococcus granulosus* G8 in Eurasia and a reappraisal of the phylogenetic relationships of 'genotypes' G5–G10. Parasitology 135, 647–654. doi:10.1017/S0031182008004198

Morgan, E.R., Clare, E.L., Jefferies, R., Stevens, J.R., 2012. Parasite epidemiology in a changing world: can molecular phylogeography help us tell the wood from the trees? Parasitology 139, 1924–1938. https://doi.org/10.1017/S0031182012001060

M'rad, S., Oudni-M'rad, M., Filisetti, D., Mekki, M., Nouri, A., Sayadi, T., Ermanno, C., Azaiez, R., Mezhoud, H., Babba, H., 2010. Molecular identification of *Echinococcus granulosus* in Tunisia: First record of the buffalo strain (G3) in human and bovine in the country. Open Vet. Sci. J. 4. https://doi.org/10.2174/1874318801004010027

Nakao, M., Lavikainen, A., Hoberg, E., 2015. Is *Echinococcus intermedius* a valid species? Trends Parasitol. 31, 342–343.

Nakao, M., Li, T., Han, X., Ma, X., Xiao, N., Qiu, J., Wang, H., Yanagida, T., Mamuti, W., Wen, H., Moro, P.L., Giraudoux, P., Craig, P.S., Ito, A., 2010. Genetic polymorphisms of *Echinococcus* tapeworms in China as determined by mitochondrial and nuclear DNA sequences. Int. J. Parasitol. 40, 379–385. doi:10.1016/j.ijpara.2009.09.006

Nakao, M., Yanagida, T., Konyaev, S., Lavikainen, A., Odnokurtsev, V.A., Zaikov, V.A., Ito, A., 2013. Mitochondrial phylogeny of the genus *Echinococcus* (Cestoda: Taeniidae) with emphasis on relationships among *Echinococcus canadensis* genotypes. Parasitology 140, 1625–1636. doi:10.1017/S0031182013000565

Nikmanesh, B., Mirhendi, H., Ghalavand, Z., Alebouyeh, M., Sharbatkhori, M., Kia, E., Mohebali, M., Eghbali, M., Rokni, M.B., 2014. Genotyping of *Echinococcus granulosus* isolates from human clinical samples based on sequencing of mitochondrial genes in Iran, Tehran. Iran. J. Parasitol. 9, 20–27.

Pednekar, R.P., Gatne, M.L., Thompson, R.C.A., Traub, R.J., 2009. Molecular and morphological characterisation of *Echinococcus* from food producing animals in India. Vet. Parasitol. 165, 58–65. https://doi.org/10.1016/j.vetpar.2009.06.021

Pezeshki, A., Akhlaghi, L., Sharbatkhori, M., Razmjou, E., Oormazdi, H., Mohebali, M., Meamar, A.R., 2013. Genotyping of *Echinococcus granulosus* from domestic animals and humans from Ardabil Province, northwest Iran. J. Helminthol. 87, 387–391. https://doi.org/10.1017/S0022149X1200051X

Possenti, A., Manzano-Román, R., Sánchez-Ovejero, C., Boufana, B., La Torre, G., Siles-Lucas, M., Casulli, A., 2016. Potential risk factors associated with human cystic echinococcosis: systematic review and meta-analysis. PLoS Negl. Trop. Dis. 10: e0005114. doi:10.1371/journal.pntd.0005114.

Rambaut, A., 2014. Figtree, a graphical viewer of phylogenetic trees. Available from http://tree.bio.ed.ac.uk/software/figtree

Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2014. Tracer v1.6, Available from http://tree.bio.ed.ac.uk/software/tracer/

Rando, J.C., Cabrera, V.M., Larruga, J.M., Hernandez, M., Gonzalez, A.M., Pinto, F., Bandelt, H.J., 1999. Phylogeographic patterns of mtDNA reflecting the colonization of the Canary Islands. Ann. Hum. Genet. 63, 413–428.

Rannamäe, E., Lõugas, L., Niemi, M., Kantanen, J., Maldre, L., Kadõrova, N., Saarma, U., 2016. Maternal and paternal genetic diversity of ancient sheep in

Estonia from the Late Bronze Age to the Post-Medieval Period, and comparison with other regions in Eurasia. Anim. Genet., doi:10.1111/age.12407
Rodero, A., Delgado, J.V., Rodero, E., 1992. Primitive Andalusian livestock and their implications in the discovery of America. Arch. Zootec. 41, 383–400.
Romig, T., Deplazes, P., Jenkins, D., Giraudoux, P., Massolo, A., Craig, P.S., Wassermann, M., Takahashi, K., de la Rue, M., 2017. Ecology and life cycle patterns of *Echinococcus* species. Adv. Parasitol. 95, 213–314. https://doi.org/10.1016/bs.apar.2016.11.002
Romig, T., Ebi, D., Wassermann, M., 2015. Taxonomy and molecular epidemiology of *Echinococcus granulosus* sensu lato. Vet. Parasitol. 213, 76–84. doi:10.1016/j.vetpar.2015.07.035
Rostami, S., Shariat Torbaghan, S., Dabiri, S., Babaei, Z., Mohammadi, M.A., Sharbatkhori, M., Fasihi Harandi, M., 2015. Genetic characterization of *Echinococcus granulosus* from a large number of formalin-fixed, paraffin-embedded tissue. Am. J. Trop. Med. Hyg. 92, 588–594. doi:10.4269/ajtmh.14-0585
Rostami Nejad, M., Taghipour, N., Nochi, Z., Mojarad, E.N., Mohebbi, S.R., Harandi, M.F., Zali, M.R., 2012. Molecular identification of animal isolates of *Echinococcus granulosus* from Iran using four mitochondrial genes. J. Helminthol. 86, 485–92. doi:10.1017/S0022149X1100071X.
Saarma, U., Ho, S.Y.W., Pybus, O.G., Kaljuste, M., Tumanov, I.L., Kojola, I., Vorobiev, A.A., Markov, N.I., Saveljev, A.P., Valdmann, H., Lyapunova, E.A., Abramov, A.V., Männil, P., Korsten, M., Vulla, E., Pazetnov, S.V., Pazetnov, V.S., Putchkovskiy, S.V., Rõkov, A.M., 2007. Mitogenetic structure of brown bears (*Ursus arctos* L.) in northeastern Europe and a new time frame for the formation of European brown bear lineages. Mol. Ecol. 16, 401–413. https://doi.org/10.1111/j.1365-294X.2006.03130.x
Saarma, U., Jõgisalu, I., Moks, E., Varcasia, A., Lavikainen, A., Oksanen, A., Simsek, S., Andresiuk, V., Denegri, G., González, L.M., Ferrer, E., Garate, T., Rinaldi, L., Maravilla, P., 2009. A novel phylogeny for the genus *Echinococcus*, based on nuclear data, challenges relationships based on mitochondrial evidence. Parasitology 136, 317–328. doi:10.1017/S0031182008005453
Santucci, F., Emerson, B.C., Hewitt, G.M., 1998. Mitochondrial DNA phylogeography of European hedgehogs. Mol. Ecol. 7, 1163–1172.
Schmitt, T., Varga, Z., 2012. Extra-Mediterranean refugia: The rule and not the exception? Front. Zool. 9, 22. https://doi.org/10.1186/1742-9994-9-22
Scott, J.C., Stefaniak, J., Pawlowski, Z.S., McManus, D.P., 1997. Molecular genetic analysis of human cystic hydatid cases from Poland: identification of a new genotypic group (G9) of *Echinococcus granulosus*. Parasitology 114, 37–43.
Sharbatkhori, M., Fasihi Harandi, M., Mirhendi, H., Hajialilo, E., Kia, E.B., 2011. Sequence analysis of cox1 and nad1 genes in *Echinococcus granulosus* G3 genotype in camels (*Camelus dromedarius*) from central Iran. Parasitol. Res. 108, 521–527. https://doi.org/10.1007/s00436-010-2092-7
Sharma, M., Fomda, B.A., Mazta, S., Sehgal, R., Bagicha Singh, B., Malla, N., 2013a. Genetic diversity and population genetic structure analysis of *Echinococcus granulosus* sensu stricto complex based on mitochondrial DNA signature. PLoS ONE 8, e82904. https://doi.org/10.1371/journal.pone.0082904
Sharma, M., Sehgal, R., Fomda, B.A., Malhotra, A., Malla, N., 2013b. Molecular characterization of *Echinococcus granulosus* cysts in north Indian patients:

identification of G1, G3, G5 and G6 genotypes. PLoS Negl. Trop. Dis. 7, e2262. https://doi.org/10.1371/journal.pntd.0002262

Šnabel, V., Altintas, N., D'Amelio, S., Nakao, M., Romig, T., Yolasigmaz, A., Gunes, K., Turk, M., Busi, M., Hüttner, M., Sevcová, D., Ito, A., Altintas, N., Dubinský, P., 2009. Cystic echinococcosis in Turkey: genetic variability and first record of the pig strain (G7) in the country. Parasitol. Res. 105, 145–154. https://doi.org/10.1007/s00436-009-1376-2

Taberlet, P., Bouvet, J., 1994. Mitochondrial DNA polymorphism, phylogeography, and conservation genetics of the brown bear *Ursus arctos* in Europe. Proc. Biol. Sci. 255, 195–200. https://doi.org/10.1098/rspb.1994.0028

Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123, 585–595.

Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10, 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023

Tappe, D., Stich, A., Frosch, M., 2008. Emergence of polycystic neotropical echinococcosis. Emerg. Infect. Dis. 14, 292–297. https://doi.org/10.3201/eid1402.070742

Tavaré, S., 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. Lect. Math. Life Sci. 17, 57–86.

Thompson, R., 2008. The taxonomy, phylogeny and transmission of *Echinococcus*. Exp. Parasitol. 119, 439–446. doi:10.1016/j.exppara.2008.04.016

Thompson, R.C.A., 2017. Biology and systematics of *Echinococcus*. Adv. Parasitol. 95, 65–109. https://doi.org/10.1016/bs.apar.2016.07.001

Thompson, R.A., McManus, D.P., 2002. Towards a taxonomic revision of the genus *Echinococcus*. Trends Parasitol. 18, 452–457. doi:10.1016/S1471-4922(02)02358-9

Torgerson, P.R., Budke, C.M., 2003. Echinococcosis – an international public health challenge. Res. Vet. Sci. 74, 191–202.

Torgerson, P.R., Macpherson, C.N.L., 2011. The socioeconomic burden of parasitic zoonoses: Global trends. Vet. Parasitol., Special issue: Zoonoses in a Changing World 182, 79–95. https://doi.org/10.1016/j.vetpar.2011.07.017

Varcasia, A., Canu, S., Kogkos, A., Pipia, A.P., Scala, A., Garippa, G., Seimenis, A., 2007. Molecular characterization of *Echinococcus granulosus* in sheep and goats of Peloponnesus, Greece. Parasitol. Res. 101, 1135–1139. https://doi.org/10.1007/s00436-007-0568-x

Varcasia, A., Tanda, B., Giobbe, M., Solinas, C., Pipia, A.P., Malgor, R., Carmona, C., Garippa, G., Scala, A., 2011. Cystic echinococcosis in Sardinia: farmers' knowledge and dog infection in sheep farms. Vet. Parasitol. 181, 335–340. doi:10.1016/j.vetpar.2011.05.006

Vural, G., Baca, A.U., Gauci, C.G., Bagci, O., Gicik, Y., Lightowlers, M.W., 2008. Variability in the *Echinococcus granulosus Cytochrome C oxidase* 1 mitochondrial gene sequence from livestock in Turkey and a re-appraisal of the G1-3 genotype cluster. Vet. Parasitol. 154, 347–350. https://doi.org/10.1016/j.vetpar.2008.03.020

Wassermann, M., Woldeyes, D., Gerbi, B.M., Ebi, D., Zeyhle, E., Mackenstedt, U., Petros, B., Tilahun, G., Kern, P., Romig, T., 2016. A novel zoonotic genotype related to *Echinococcus granulosus* sensu stricto from southern Ethiopia. Int. J. Parasitol. 46, 663–668. https://doi.org/10.1016/j.ijpara.2016.04.005

World Health Organization, 2012. Research priorities for helminth infections: technical report of the TDR Disease Reference group on Helminth Infections, Geneva.

World Health Organization, 2015. Investing to overcome the global impact of neglected tropical diseases: Third WHO report on neglected tropical diseases, Geneva.

Yan, N., Nie, H.-M., Jiang, Z.-R., Yang, A.-G., Deng, S.-J., Guo, L., Yu, H., Yan, Y.-B., Tsering, D., Kong, W.-S., 2013. Genetic variability of *Echinococcus granulosus* from the Tibetan plateau inferred by mitochondrial DNA sequences. Vet. Parasitol. 196, 179–183. doi:10.1016/j.vetpar.2013.02.010

Yanagida, T., Lavikainen, A., Hoberg, E.P., Konyaev, S., Ito, A., Sato, M.O., Zaikov, V.A., Beckmen, K., Nakao, M., 2017. Specific status of *Echinococcus canadensis* (Cestoda: Taeniidae) inferred from nuclear and mitochondrial gene sequences. Int. J. Parasitol. 47, 971–979. https://doi.org/10.1016/j.ijpara.2017.07.001

Yanagida, T., Mohammadzadeh, T., Kamhawi, S., Nakao, M., Sadjjadi, S.M., Hijjawi, N., Abdel-Hafez, S.K., Sako, Y., Okamoto, M., Ito, A., 2012. Genetic polymorphisms of *Echinococcus granulosus* sensu stricto in the Middle East. Parasitol. Int. 61, 599–603. doi:10.1016/j.parint.2012.05.014

Yang, Z., 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. J. Mol. Evol. 39, 306–314.

Yindee, M., Vlamings, B.H., Wajjwalku, W., Techakumphu, M., Lohachit, C., Sirivaidyapong, S., Thitaram, C., Amarasinghe, A.A., Alexander, P.A., Colenbrander, B., Lenstra, J.A., 2010. Y-chromosomal variation confirms independent domestications of swamp and river buffalo. Anim. Genet. 41, 433–435. https://doi.org/10.1111/j.1365-2052.2010.02020.x

Zait, H., Kouidri, M., Grenouillet, F.E., Umhang, G., Millon, L., Hamrioui, B., Grenouillet, F., 2016. Molecular characterization of *Echinococcus granulosus* sensu stricto and *Echinococcus canadensis* in humans and livestock from Algeria. Parasitol. Res. 115, 2423–2431. https://doi.org/10.1007/s00436-016-4994-5

Zeder, M.A., 2008. Domestication and early agriculture in the Mediterranean Basin: Origins, diffusion, and impact. Proc. Natl. Acad. Sci. 105, 11597–11604. doi:10.1073/pnas.0801317105

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Kinkar, L., Laurimäe, T., Acosta-Jamett, G., Andresiuk, V., Balkaya, I., Casulli, A., Gasser, R.B., van der Giessen, J., González, LM., Haag, K.L., Zait, H., Irshadullah, M., Jabbar, A., Jenkins, D.J., Kia, E.B., Manfredi, M.T., Mirhendi, H., M'rad, S., Rostami-Nejad, M., Oudni-M'rad, M., Pierangeli, N.B., Ponce-Gordo, F., Rehbein, S., Sharbatkhori, M., Simsek, S., Soriano, S.V., Sprong, H., Šnábel, V., Umhang, G., Varcasia, A., Saarma, U. (2018). Global phylogeography and genetic diversity of the zoonotic tapeworm *Echinococcus granulosus* sensu stricto genotype G1. ***International Journal for Parasitology***. *In Press*.

Kinkar, L., Laurimäe, T., Balkaya, I., Casulli, A., Houria, Z., Irshadullah, M., Sharbatkhori, M., Mirhendi, H., Rostami Nejad, M., Ponce-Gordo, F., Rehbein, S., Kia, E.B., Simsek, S., Šnábel, V., Umhang, G., Varcasia, A., Saarma, U. (2018). Genetic diversity and phylogeography of the elusive, but epidemiologically important *Echinococcus granulosus* sensu stricto genotype G3. ***Parasitology***. *In Press*.

Laurimäe, T., **Kinkar, L.**, Romig, T., Omer, R.A., Casulli, A., Umhang, G., Gasser, R., Jabbar, A., Sharbatkori, M., Mirhendi, H., Ponce-Gordo, F., Lazzarini, L., Soriano, S.V., Varcasia, A., Rostami-Nejad, M., Andresiuk, V., Maravilla, P., González, L., Dybicz, M., Gawor, J., Šarkunas, M., Snabel, V., Kuzmina, T., Saarma, U. (2018). The benefits of analysing complete mitochondrial genomes: deep insights into the phylogeny and population structure of *Echinococcus granulosus* sensu lato genotypes G6 and G7. ***Infection, Genetics and Evolution***. *In Press*.

Laurimäe, T., **Kinkar, L.**, Moks, E., Romig, T., Omer, R.A., Casulli, A., Umhang, G., Bagrade, G., Irshadullah, M., Sharbatkhori, M., Mirhendi, H., Ponce-Gordo, F., Soriano, S.V., Varcasia, A., Rostami-Nejad, M., Andresiuk, V., Saarma, U. (2018). Molecular phylogeny based on six nuclear genes suggests that *Echinococcus granulosus* sensu lato genotypes G6/G7 and G8/G10 can be regarded as two distinct species. ***Parasitology***. *In Press*.

Kinkar, L., Laurimäe, T., Sharbatkhori, M., Mirhendi, H., Kia, E.B., Ponce-Gordo, F., Andresiuk, V., Simsek, S., Lavikainen, A., Irshadullah, M., Umhang, G., Oudni-M'rad, M., Acosta-Jamett, G., Rehbein, S., Saarma, U. (2017). New mitogenome and nuclear evidence on the phylogeny and taxonomy of the highly zoonotic tapeworm *Echinococcus granulosus* sensu stricto. ***Infection, Genetics and Evolution***, 52, 52−58.

Kinkar, L., Laurimäe, T., Simsek, S., Balkaya, I., Casulli, A., Manfredi, M.T., Ponce-Gordo, F., Varcasia, A., Lavikainen, A., González, L.M., Rehbein, S., van der Giessen, J., Sprong, H., Saarma, U. (2016). High-resolution phylogeography of zoonotic tapeworm *Echinococcus granulosus* sensu stricto genotype G1 with an emphasis on its distribution in Turkey, Italy and Spain. ***Parasitology***, 143, 1790−1801.

Laurimäe, T., **Kinkar, L.**, Andresiuk, V., Haag, K.L., Ponce-Gordo, F., Acosta-Jamett, G., Garate, T., González, L.M., Saarma, U. (2016). Genetic diversity and phylogeography of highly zoonotic *Echinococcus granulosus* genotype G1 in the Americas (Argentina, Brazil, Chile and Mexico) based on 8279 bp of mtDNA. ***Infection, Genetics and Evolution***, 45, 290−296.

Laurimaa, L., Davison, J., Süld, K., Plumer, L., Oja, R., Moks, E., Keis, M., Hindrikson, M., **Kinkar, L.**, Laurimäe, T., Abner, J., Remm, J., Anijalg, P., Saarma, U. (2015). First report of highly pathogenic *Echinococcus granulosus* genotype G1 in dogs in European urban environment. ***Parasites & Vectors***, 8, 182.

Laurimaa, L., Davison, J., Plumer, L., Süld, K., Oja, R., Moks, E., Keis, M., Hindrikson, M., **Kinkar, L.**, Laurimäe, T., Abner, J., Remm, J., Anijalg, P., Saarma, U. (2015). Noninvasive detection of *Echinococcus multilocularis* tapeworm in urban area, Estonia. ***Emerging Infectious Diseases***, 21, 163−164.

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Kinkar, L., Laurimäe, T., Acosta-Jamett, G., Andresiuk, V., Balkaya, I., Casulli, A., Gasser, R.B., van der Giessen, J., González, LM., Haag, K.L., Zait, H., Irshadullah, M., Jabbar, A., Jenkins, D.J., Kia, E.B., Manfredi, M.T., Mirhendi, H., M'rad, S., Rostami-Nejad, M., Oudni-M'rad, M., Pierangeli, N.B., Ponce-Gordo, F., Rehbein, S., Sharbatkhori, M., Simsek, S., Soriano, S.V., Sprong, H., Šnábel, V., Umhang, G., Varcasia, A., Saarma, U. (2018). Global phylogeography and genetic diversity of the zoonotic tapeworm *Echinococcus granulosus* sensu stricto genotype G1. ***International Journal for Parasitology***. *In Press*.

Kinkar, L., Laurimäe, T., Balkaya, I., Casulli, A., Houria, Z., Irshadullah, M., Sharbatkhori, M., Mirhendi, H., Rostami Nejad, M., Ponce-Gordo, F., Rehbein, S., Kia, E.B., Simsek, S., Šnábel, V., Umhang, G., Varcasia, A., Saarma, U. (2018). Genetic diversity and phylogeography of the elusive, but

epidemiologically important *Echinococcus granulosus* sensu stricto genotype G3. ***Parasitology***. *In Press*.

Laurimäe, T., **Kinkar, L.**, Romig, T., Omer, R.A., Casulli, A., Umhang, G., Gasser, R., Jabbar, A., Sharbatkori, M., Mirhendi, H., Ponce-Gordo, F., Lazzarini, L., Soriano, S.V., Varcasia, A., Rostami-Nejad, M., Andresiuk, V., Maravilla, P., González, L., Dybicz, M., Gawor, J., Šarkunas, M., Snabel, V., Kuzmina, T., Saarma, U. (2018). The benefits of analysing complete mitochondrial genomes: deep insights into the phylogeny and population structure of *Echinococcus granulosus* sensu lato genotypes G6 and G7. ***Infection, Genetics and Evolution***. *In Press*.

Laurimäe, T., **Kinkar, L.**, Moks, E., Romig, T., Omer, R.A., Casulli, A., Umhang, G., Bagrade, G., Irshadullah, M., Sharbatkhori, M., Mirhendi, H., Ponce-Gordo, F., Soriano, S.V., Varcasia, A., Rostami-Nejad, M., Andresiuk, V., Saarma, U. (2018). Molecular phylogeny based on six nuclear genes suggests that *Echinococcus granulosus* sensu lato genotypes G6/G7 and G8/G10 can be regarded as two distinct species. ***Parasitology***. *In Press*.

Kinkar, L., Laurimäe, T., Sharbatkhori, M., Mirhendi, H., Kia, E.B., Ponce-Gordo, F., Andresiuk, V., Simsek, S., Lavikainen, A., Irshadullah, M., Umhang, G., Oudni-M'rad, M., Acosta-Jamett, G., Rehbein, S., Saarma, U. (2017). New mitogenome and nuclear evidence on the phylogeny and taxonomy of the highly zoonotic tapeworm *Echinococcus granulosus* sensu stricto. ***Infection, Genetics and Evolution***, 52, 52−58.

Kinkar, L., Laurimäe, T., Simsek, S., Balkaya, I., Casulli, A., Manfredi, M.T., Ponce-Gordo, F., Varcasia, A., Lavikainen, A., González, L.M., Rehbein, S., van der Giessen, J., Sprong, H., Saarma, U. (2016). High-resolution phylogeography of zoonotic tapeworm *Echinococcus granulosus* sensu stricto genotype G1 with an emphasis on its distribution in Turkey, Italy and Spain. ***Parasitology***, 143, 1790−1801.

Laurimäe, T., **Kinkar, L.**, Andresiuk, V., Haag, K.L., Ponce-Gordo, F., Acosta-Jamett, G., Garate, T., González, L.M., Saarma, U. (2016). Genetic diversity and phylogeography of highly zoonotic *Echinococcus granulosus* genotype G1 in the Americas (Argentina, Brazil, Chile and Mexico) based on 8279 bp of mtDNA. ***Infection, Genetics and Evolution***, 45, 290−296.

Laurimaa, L., Davison, J., Süld, K., Plumer, L., Oja, R., Moks, E., Keis, M., Hindrikson, M., **Kinkar, L.**, Laurimäe, T., Abner, J., Remm, J., Anijalg, P., Saarma, U. (2015). First report of highly pathogenic *Echinococcus granulosus* genotype G1 in dogs in European urban environment. ***Parasites & Vectors***, 8, 182.

Laurimaa, L., Davison, J., Plumer, L., Süld, K., Oja, R., Moks, E., Keis, M., Hindrikson, M., **Kinkar, L.**, Laurimäe, T., Abner, J., Remm, J., Anijalg, P., Saarma, U. (2015). Noninvasive detection of *Echinococcus multilocularis* tapeworm in urban area, Estonia. ***Emerging Infectious Diseases***, 21, 163−164.

Konverentsiettekanded:

Kinkar, L., Laurimäe, T., Saarma, U. New mitogenome and nuclear evidence on the phylogeny and taxonomy of the highly zoonotic tapeworm *Echinococcus granulosus* sensu stricto. 10th Baltic Theriological Conference, 27–30.09.2017, Tartu, Estonia. Suuline ettekanne.

Kinkar, L., Laurimäe, T., Saarma, U. Phylogeny and taxonomy of the *Echinococcus granulosus* complex: genotypes G1-G3 and G6-G7. 12th European Multicolloquium of Parasitology, 20–24.07.2016, Turku, Finland. Suuline ettekanne.

Kinkar, L., Laurimäe, T., Saarma, U. Mitochondrial variation of *Echinococcus granulosus* genotype G1 in Europe. 6th Conference of the Scandinavian-Baltic Society for Parasitology, 22.–24.04.2015, Uppsala, Sweden. Suuline ettekanne.

Kursused:

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