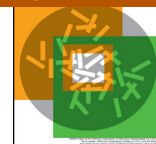




## Veterinary Parasitology

journal homepage: [www.elsevier.com/locate/vetpar](http://www.elsevier.com/locate/vetpar)Taxonomy and molecular epidemiology of *Echinococcus granulosus* sensu lato

T. Romig\*, D. Ebi, M. Wassermann

Universität Hohenheim, FG Parasitologie 220 B, 70599 Stuttgart, Germany

## ARTICLE INFO

## Keywords:

*Echinococcus granulosus*

Taxonomy

Nomenclature

Molecular epidemiology

## ABSTRACT

*Echinococcus granulosus*, formerly regarded as a single species with a high genotypic and phenotypic diversity, is now recognised as an assemblage of cryptic species, which differ considerably in morphology, development, host specificity (including infectivity/pathogenicity for humans) and other aspects. This diversity is reflected in the mitochondrial and nuclear genomes and has led to the construction of phylogenetic trees and hypotheses on the origin and geographic dispersal of various taxa. Based on phenotypic characters and gene sequences, *E. granulosus* (sensu lato) has by now been subdivided into *E. granulosus* sensu stricto (including the formerly identified genotypic variants G1–3), *Echinococcus felidis* (the former 'lion strain'), *Echinococcus equinus* (the 'horse strain', genotype G4), *Echinococcus ortleppi* (the 'cattle strain', genotype G5) and *Echinococcus canadensis*. The latter species, as recognised here, shows the highest diversity and is composed of the 'camel strain', genotype G6, the 'pig strain', genotype G7, and two 'cervid strains', genotypes G8 and G10. There is debate whether the closely related G6 and G7 should be placed in a separate species, but more morphological and biological data are needed to support or reject this view. In this classification, the application of rules for zoological nomenclature led to the resurrection of old species names, which had before been synonymised with *E. granulosus*. This nomenclatural subdivision of the agents of cystic echinococcosis (CE) may appear inconvenient for practical applications, especially because molecular tools are needed for identification of the cyst stage, and because retrospective data on '*E. granulosus*' are now difficult to interpret without examination of voucher specimens. However, the increased awareness for the diversity of CE agents – now emphasised by species names rather than genotype numbers – has led to a large number of recent studies on this issue and a rapid increase of knowledge on geographical spread, host range and impact on human health of the various species. *E. granulosus* s.s., often transmitted by sheep, is now clearly identified as the principal CE agent affecting humans. Contrary to previous assumptions, genotypes G6/7 of *E. canadensis* readily infect humans, although CE incidences are rather low where *E. canadensis* predominates. Sub-Saharan Africa seems to be the region with the highest diversity of *Echinococcus*, and wild carnivores may play a more important role in the lifecycles of various species than previously assumed. Still, a number of issues remain unclear, e.g. possibly diverging parameters of diagnostic tests among the species, different responses to vaccines and, importantly, possibly required modifications of clinical management due to differences in pathogenicity.

© 2015 Elsevier B.V. All rights reserved.

## 1. A summarised history of *Echinococcus* nomenclature and taxonomy

### 1.1. Early period

Echinococcosis of humans and livestock has been known – and named in various languages – since antiquity. After the introduction of modern zoological nomenclature in 1758, intended to bring order into the infinite number of poorly defined local names for animals, naming of what we define today as *Echinococcus* spp. became rather chaotic initially. No less than 85 bi- or trinomial

\* Corresponding author at: Universität Hohenheim, FG Parasitologie 220 B, Emil-Wolff-Str. 34, 70599 Stuttgart, Germany. Fax: +49 711 459 22276.

E-mail addresses: [Thomas.Romig@uni-hohenheim.de](mailto:Thomas.Romig@uni-hohenheim.de) (T. Romig),

[Dennis.Ebi@uni-hohenheim.de](mailto:Dennis.Ebi@uni-hohenheim.de) (D. Ebi), [Marion.Wassermann@uni-hohenheim.de](mailto:Marion.Wassermann@uni-hohenheim.de) (M. Wassermann).

**Table 1**Synopsis of relevant descriptions of species and subspecies. Agents of cystic echinococcosis (*E. granulosus sensu lato*) in bold print.

Original name (description)	Original description from (stage, host, country)	Current name
<b><i>Hydatigena granulosus</i> Batsch (1796)</b>	Metacestode, sheep, Germany	<i>Echinococcus granulosus</i>
<i>Taenia multilocularis</i> Leuckart (1863)	Metacestode, human, Germany	<i>E. multilocularis</i>
<i>T. oligarthra</i> Diesing (1863)	Adult, puma, Brazil	<i>E. oligarthra</i>
<i>Echinococcus cruzi</i> Brumpt and Joyeux (1924)	Metacestode, agouti, Brazil	<i>E. oligarthra</i>
<b><i>E. minimus</i> Cameron (1926)</b>	Adult, wolf, Europe	<i>E. granulosus</i>
<b><i>E. longimanubrius</i> Cameron (1926)</b>	Adult, African wild dog, South Africa	<i>E. granulosus</i>
<b><i>E. cameroni</i> Ortlepp (1934)</b>	Adult, red fox, Britain	<i>E. granulosus</i>
<b><i>E. lycaontis</i> Ortlepp (1934)</b>	Adult, African wild dog <sup>a</sup> , South Africa	<i>E. granulosus</i>
<b><i>E. felidis</i> Ortlepp (1937)</b>	Adult, lion, South Africa	<i>E. felidis</i>
<b><i>E. intermedius</i> Lopez-Neyra and Soler Planas (1943)</b>	Adult, dog, Spain	<i>E. granulosus</i> <sup>b</sup>
<b><i>E. ortleppi</i> Lopez-Neyra and Soler Planas (1943)</b>	Adult, dog, South Africa	<i>E. ortleppi</i>
<i>E. sibiricensis</i> Rausch and Schiller (1954)	Adult, arctic fox, St. Lawrence Isl.	<i>E. multilocularis</i>
<b><i>E. patagonicus</i> Szidat (1960)</b>	Adult, Lycalopex, Argentina	<i>E. granulosus</i>
<b><i>E. granulosus canadensis</i> Webster and Cameron (1961)</b>	Metacestode <sup>e</sup> , reindeer, Canada	<i>E. canadensis</i> <sup>b</sup>
<b><i>E. granulosus borealis</i> Sweatman and Williams (1963)</b>	Metacestode <sup>e</sup> , moose, Canada	<i>E. canadensis</i> <sup>b</sup>
<b><i>E. granulosus equinus</i> Williams and Sweatman (1963)</b>	Adult, dog <sup>d</sup> , Britain	<i>E. equinus</i>
<b><i>E. granulosus africanus</i> Verster (1965)</b>	Adult <sup>e</sup> , div. Canidae, South Africa	<i>E. granulosus</i>
<i>E. pampeanus</i> Szidat (1967)	Adult, <i>Leopardus colocolo</i> , Argentina	<i>E. oligarthra</i>
<b><i>E. granulosus dusicyontis</i> Blood and Lelijveld (1969)</b>	Adult, <i>Lycalopex</i> , Argentina	<i>E. granulosus</i>
<b><i>E. cepanzoi</i> Szidat (1971)</b>	Adult, <i>Lycalopex</i> , Argentina	<i>E. granulosus</i>
<i>E. vogeli</i> Rausch and Bernstein (1972)	Adult, bush dog, Ecuador	<i>E. vogeli</i>
<i>E. shiquicus</i> Xiao et al. (2005)	Adult, Tibetan fox, China	<i>E. shiquicus</i>
<i>E. russicensis</i> Tang et al. (2007)	Adult, corsac fox, China	<i>E. multilocularis</i>

<sup>a</sup> From cyst of sheep origin.<sup>b</sup> Species status under evaluation.<sup>c</sup> Supplemented by adult worms from experimental infections.<sup>d</sup> From cyst of horse origin.<sup>e</sup> From cyst of cattle origin.

Latinised names were published until the end of the 19th century, almost all of them based on metacestodes of various morphological appearance and host origin (Abuladze, 1964). The first valid name of these was *Hydatigena granulosus*, given by Batsch in 1786 and recognisably based on a fertile *Echinococcus* cyst of sheep origin from Germany. Shortly after, Rudolphi established the genus *Echinococcus* in 1801, the name referring to the small, round, 'spiny' protoscolices found in the cysts, and thus created the combination *E. granulosus*, which is still in use today. Not recognising the link between metacestodes and adult worms, Rudolphi described adult *Echinococcus* from a dog as *Taenia cateniformis* in 1808. An additional description of adult worms was provided by Beneden in 1856, as *Taenia nana*, in ignorance of the fact that three years earlier the relationship between cysts and adult worms in dogs had already been proven after independent feeding experiments by von Siebold and Küchenmeister. Eventually, at the end of the 19th century, the common name *E. granulosus* referred to all stages of the lifecycle, although synonyms like *Taenia echinococcus* remained in use for a long time after. Despite the large number of names that had been given to cysts due to their morphological appearances, echinococcosis eventually was commonly assumed to be caused by a single species. Even the metacestode of alveolar echinococcosis (described as *Echinococcus multilocularis* Leuckart, 1863) with its extremely divergent morphology and pathology in humans, and its peculiar geographical restriction, was viewed by the majority of authors (the 'unicists') as a modification of *E. granulosus*. The 'dualists' – postulating a different species causing this disease – were in defensive position until the 1950s, when the lifecycle of *E. multilocularis* was discovered almost simultaneously on St. Lawrence Island off Alaska and in central Europe (lit. in Abuladze, 1964; Tappe et al., 2010).

## 1.2. Species

Despite the debate whether echinococcosis might be caused by one or by two species, a substantial number of additional *Echinococcus* species had meanwhile been described based on adult worm

morphology, e.g. differences in the number of proglottids, rostellar hook morphology, number and distribution of testes and position of the genital pore. In addition to *E. granulosus* (Batsch, 1786) and *E. multilocularis* Leuckart, 1863 – whose descriptions were based on metacestodes – *Echinococcus oligarthra*<sup>1</sup> was described by Diesing (as *Taenia*) as early as 1863 (later, the metacestode was separately described under the synonym *E. cruzi* Brumpt and Joyeux, 1924). *Echinococcus minimus* and *Echinococcus longimanubrius* were described by Cameron (1926) from a European (Macedonian) wolf and an African wild dog, respectively (Brumpt and Joyeux, 1924; Cameron, 1926; Diesing, 1863). Ortlepp added *Echinococcus cameroni* (for worms from a British fox that Cameron had identified earlier as *E. granulosus*) and *Echinococcus lycaontis* (from an African wild dog), followed by *Echinococcus felidis* from an African lion (Ortlepp, 1934; Ortlepp, 1937). *Echinococcus sibiricensis* Rausch and Schiller, 1954 was shortly after description synonymised *E. multilocularis* (Vogel, 1955; Vogel, 1957). This was followed by the descriptions of *E. intermedius* and *E. ortleppi* (Lopez-Neyra and Soler Planas, 1943) from domestic dogs in Spain and South Africa, respectively, and *Echinococcus patagonicus* (Szidat, 1960), from a wild South American canid (*Lycalopex culpaesus*) (Lopez-Neyra and Soler Planas, 1943; Szidat, 1960). However, in a concise evaluation of published morphological data, Rausch and Nelson sank most of these names as synonyms under *E. granulosus*, mainly on grounds of uncertainty about the extent of variability of the diagnostic characters used (Rausch and Nelson, 1963). Only two additional species, *E. multilocularis* and *E. oligarthra*, were considered valid by these authors, while *E. felidis* and *E. patagonicus* were given uncertain status awaiting further data. A further five species were described later on, of which *E. pampeanus* Szidat, 1967 and *E. cepanzoi* Szidat, 1971 were synonymised with *E. oligarthra* and *E. granulosus*, respectively (Schantz et al., 1976), and *E. russicensis* Tang et al., 2007 is now thought to be a variant of *E. multilocularis* (Nakao et al., 2013a).

<sup>1</sup> For the spelling of *Echinococcus oligarthra* (vs. *E. oligarthrus*) see Hüttner and Romig, 2009 and Nakao et al.

**Table 2**  
Current concept of *Echinococcus* taxonomy (agents of cystic echinococcosis in bold).

Species	Genotypes and strains
<i>Echinococcus granulosus</i> Batsch (1796)	G1-3, sheep / buffalo strains
<i>E. equinus</i> Williams and Sweatman (1963)	G4, horse strain
<i>E. ortleppi</i> Lopez-Neyra and Soler Planas (1943)	<b>G5, cattle strain</b>
<i>E. canadensis</i> Webster and Cameron (1961)	G6-7, camel-pig strain G8, American cervid strain G10, Fennoscandian cervid strain
<i>E. felidis</i> Ortlepp (1937)	lion strain
<i>E. multilocularis</i> Leuckart (1863)	
<i>E. shiquicus</i> Xiao et al. (2005)	
<i>E. oligarthra</i> Diesing (1863)	
<i>E. vogeli</i> Rausch and Bernstein (1972)	

Only *E. vogeli* Rausch and Bernstein, 1972 and *E. shiquicus* Xiao et al., 2005 are now considered to be valid species (Nakao et al., 2013a) (Table 1). Their phylogenetic relationships, based on four mitochondrial genes (cox1, nad1, cob, rrn), are illustrated in Fig. 1

### 1.3. Subspecies

In addition to species, various subspecies of *E. multilocularis* and *E. granulosus* were described, mostly based on worm morphology. *E. multilocularis* and *E. granulosus* were subsequently divided into various subspecies, again largely based on morphological characters of the worms. This started in 1957, when Vogel sank *E. sibiricus*, described only three years earlier by Rausch and Schiller, as a subspecies under *E. multilocularis* (a third subspecies *E. m. kazakhstanensis* Shul'ts, 1962 was described from metacestodes in ungulates) (Rausch and Schiller, 1954; Shul'ts, 1962; Vogel, 1957). *E. g. canadensis* Webster and Cameron (1961) was erected due to host preference (reindeer) of the cyst stage. Retaining this, Sweatman and Williams added *E. g. borealis* (moose – canid cycle) and *E. g. equinus* (horse – dog cycle) in addition to the nominate *E. g. granulosus* from domestic sheep, cattle and pigs (Sweatman and Williams, 1963; Williams and Sweatmen, 1963). In her major taxonomic revision of 1965, Verster retained the subspecies *borealis* and *canadensis*, resurrected *felidis*, *lycaontis* and *ortleppi* – now as subspecies –, described a new subspecies *africanus* (from cattle, sheep and dogs in South Africa), changed the designation of Sweatman and Williams' nominate subspecies to *E. g. newzealandensis*, and assigned nominate subspecies status to worm material from Germany (from where the type species had been described) (Verster, 1965). This amounted to eight subspecies of *E. granulosus* (the taxa *equinus*, *cameroni*, *intermedius*, *longimanubrius*, *minimus*, *patagonicus* and *oligarthra* were not included in the revision). Shortly after, in his largely theoretical treatment of infraspecific categories in *Echinococcus*, Rausch (1967) refuted the subspecies status for sympatric forms of *E. granulosus*, and attributed the morphological differences found by previous authors to host-induced modifications (Rausch, 1967). A possible exception to this was seen in the distinction between a 'northern form' (or *E. g. canadensis*), transmitted in wildlife cycles in North America and northern Eurasia, and a domestic form (*E. g. granulosus*), which had been globally distributed through human activities. Yet, shortly after this consolidation, three additional species were described from South America: *E. pampeanus* Szidat (1967) from a wild cat species, *E. cepanzoi* Szidat (1971) (as a new name for the subspecies *E. g. dusicyontis* Blood and Lelijveld, 1969) from a wild South American canid (*Lycalopex* sp.), and finally *E. vogeli* Rausch and Bernstein (1972); from the bush dog (*Speothos venaticus*) (Blood and Lelijveld, 1969; Rausch and Bernstein, 1972; Szidat, 1967; Szidat, 1971). Of these, only *E. vogeli* survived the test of time, while the first two

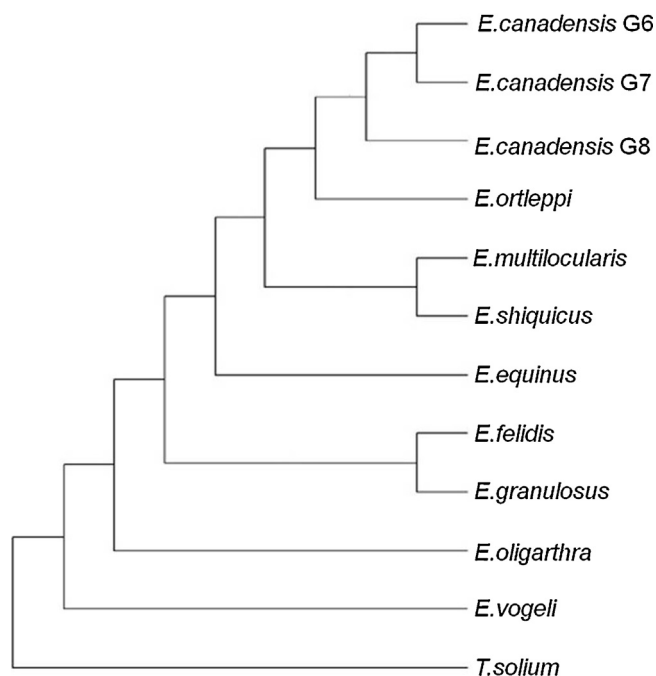
were subsequently synonymised with *E. oligarthra* and *E. granulosus*, respectively (Schantz et al., 1976).

### 1.4. Strains and genotypes

At the beginning of the 1980s, there were finally four undisputed species (*E. granulosus*, *E. multilocularis*, *E. oligarthra* and *E. vogeli*) (Kumaratilake and Thompson, 1982). It was clear, however, that *E. granulosus* contained a substantial number of variants with differences concerning morphology, host specificity, biochemical parameters, developmental biology and geographical distribution. Although the application of the biological species concept by Rausch (1967), which had led to the abolishment of all sympatric subspecies, had received criticism (Beveridge, 1974), no attempt was made to resurrect subspecies names for these variants. Instead, an informal system of intraspecific 'strains' was gradually established. This term was used to describe variants that differed from each other in characters of epidemiological significance (Thompson and McManus, 2001). Fully developed, the system comprised eleven strains, namely sheep, Tasmanian sheep, buffalo, horse, cattle, camel, pig, variant pig (or human-pig), American cervid, Fennoscandian cervid and lion strain. Originally, the strain system was based on non-genetic characters like host spectrum, geography, morphology and aspects of development. From the early 1990s, gene sequence data became increasingly important to define and identify the strains. Important contributions to the consolidation of these infraspecific categories were the publications of partial sequences of the mitochondrial cox1 and nad1 genes for seven strains of *E. granulosus*, plus *E. multilocularis*, *E. vogeli* and *E. oligarthra* (Bowles et al., 1992; Bowles and McManus, 1993a). Sequence data corresponded well to other characters defining the strains, and led to a genotype 'nomenclature' (G1 to G7), partly replacing the previous strain names. Although only a limited number of isolates from biologically or epidemiologically characterised strains were genotyped, the terms 'strain' and 'genotype' were increasingly treated as synonyms. In addition to the seven genotypes/strains that were initially characterised (G1/sheep strain; G2/Tasmanian sheep strain; G3/buffalo strain; G4/horse strain; G5/cattle strain; G6/camel strain; G7/pig strain), over time three additional taxa were added: the American cervid strain (G8) (Bowles et al., 1994), a variant pig (or human-pig) strain (G9) (Scott et al., 1997), and finally the Fennoscandian cervid strain (G10) (Lavikainen et al., 2003). Lack of material for sequencing kept the lion strain from being included in the 'G-system'.

### 1.5. Species, once more

After two decades of accumulating epidemiological, biochemical and geographic data on the *E. granulosus* strains, and the phylogenetic evaluation of increasingly long mitochondrial and nuclear gene sequences (including complete mitochondrial genomes), limitations and contradictions of the strain/genotype system within *E. granulosus* became apparent and called for a taxonomic revision of the genus. Major points were (1) the apparent paraphyly of *E. granulosus* (*sensu lato*) with respect to *E. multilocularis* and its sister taxon *E. shiquicus* (which had in the meantime been described from the Tibetan plateau; Xiao et al., 2005, 2006), and (2) the fact that genetic distances between some genotypes (G1-3, G6-7) were in the range of microvariants of the same taxon, whereas others (G4, G5) were only distantly related. After a first proposition to subdivide *E. granulosus* into four species (Thompson et al., 1995), *E. granulosus equinus* Williams and Sweatman, 1963 was finally elevated to species rank (for the horse strain, genotype G4), and *E. ortleppi* Lopez-Neyra and Soler Planas, 1943 was reinstated (for the cattle strain, genotype G5; Thompson and McManus, 2002). This left the name *E. granulosus* (Batsch, 1786) for the



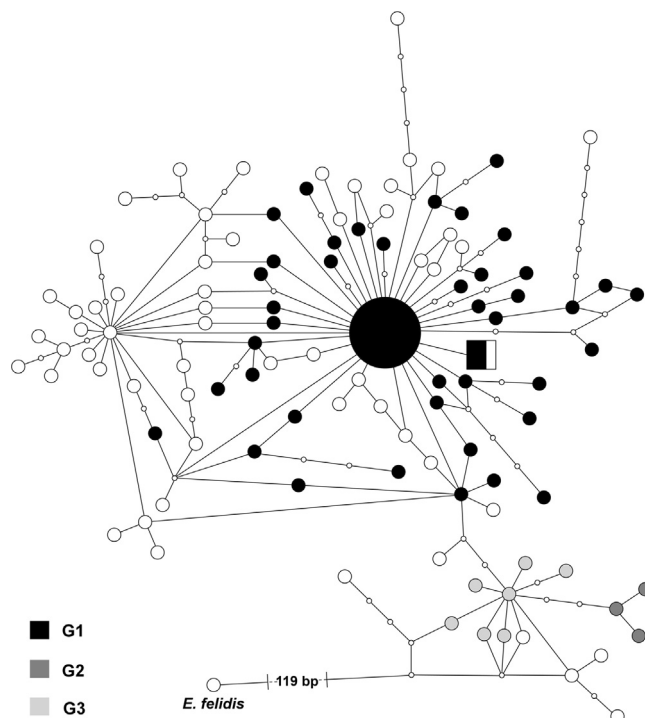
**Fig. 1.** Cladogram of *Echinococcus* spp. obtained through Maximum Likelihood analyses of 5170 nucleotides of the mitochondrial *cox1*, *nad1*, *rrn* and *cob* gene. Modified from Hüttner et al. (2008).

genotypes G1 to G3 (sheep, Tasmanian sheep and buffalo strains), while the allocation of the camel, pig, cervid and lion strains was left unresolved until, five years later and based on the comparison of complete mitochondrial genomes, *E. granulosus canadensis* Webster and Cameron, 1961 was given species status, now including several closely related genotypes (G6 to G10) (Nakao et al., 2007). Finally, *E. felidis* Ortlepp, 1937; could be resurrected from synonymy based on mitochondrial sequences obtained from preserved adult worm material that had been determined by Verster in South Africa (Hüttner et al., 2008). In the current state of this (ongoing) taxonomic reshuffle, *E. granulosus* in its previous sense (or *sensu lato*) is split into five species (*granulosus s.s.*, *felidis*, *equinus*, *ortleppi*, *canadensis*), in addition to the agents of alveolar and polycystic echinococcosis (*E. multilocularis*, *shiquicus*, *oligarthra*, *vogeli*) (Table 2).

## 2. Species accounts and molecular epidemiology

### 2.1. *Echinococcus granulosus (sensu stricto)*

The type specimen of *E. granulosus* originated from a sheep, and it is likely that it belonged to what was later known as the ‘sheep strain’ (this is less clear for Verster’s subspecies *E. g. granulosus*, as the worms used for the description originated from a pig cyst). In the current concept, this name is reserved for the ‘sheep strain’, ‘Tasmanian sheep strain’ and ‘buffalo strain’ (which correspond to the genotypes G1, G2 and G3, respectively), as well as a large number of other closely related variants. It was already apparent from the analysis of relatively short gene sequences (Bowles et al., 1992; Bowles et al., 1992a), that G1–3 were much more closely related to each other than to any other known genotype. This was confirmed by more recent studies using longer sequences and/or other genes, which also identified a large number of additional haplotypes belonging to this cluster (Busi et al., 2007; Casulli et al., 2012; Nakao et al., 2013a; Snabel et al., 2009; Vural et al., 2008; Yanagida et al., 2012). In view of this, and regarding the fact that, strictly speaking, the ‘G-nomenclature’ is defined by the short *cox1* and



**Fig. 2.** *E. granulosus sensu stricto*: haplotype network of the complete mitochondrial *cox1* gene (1608 bp). The network consists of 137 haplotypes based on 304 analysed isolates from Europe (16), western Asia (140), southern/eastern Asia (40), Africa (78) and South America (30) (authors, unpublished data and Genbank entries). The network includes haplotypes published by Konyaev et al. (2013) and Yanagida et al. (2012). For comparison, one isolate of *E. felidis* is included (Genbank accession no. AB732958). The network was constructed using TCS 1.21 (Clement et al., 2000), with fixed connection limit at 130 steps.

Circle sizes are not proportional to the haplotype frequencies. Large central circle: represents the most common, globally distributed haplotype (e.g. accession no. JQ250806). Medium circles: identified haplotypes. Small circles: hypothetical intermediate haplotypes (not identified in this panel). Rectangle: represents 28 different haplotypes with one basepair difference to the central haplotype. Black circles: haplotypes that contain the G1 sequence of Bowles et al. (1992) (21 of 28 haplotypes of the rectangle belong to this type). Dark grey circle: haplotypes that contain the G2 sequence of Bowles et al. (1992). Light grey circles: haplotypes that contain the G3 sequence of Bowles et al. (1992). White circles: not described in the G-system of genotypes.

*nad1* sequences published in the 1990s, the use of the G numbers has become increasingly misleading (see also discussion on this by Nakao et al., 2013a). A substantial number of different gene sequences have in the past been allocated to ‘G1’ or ‘G3’ without a clear definition. The problem of allocating haplotypes based on longer (or other) genome fragments is illustrated in Fig. 2. Using the complete sequence of the mitochondrial *cox1* gene, 137 haplotypes were identified among 304 isolates of the G1–3 cluster from western, eastern and southern Asia, Europe, Africa and South America. Using the original G-definition of *cox1* (366 bp sequences), a large proportion of the haplotypes are not homologous with either G1, 2 or 3, although they clearly belong to the same cluster. Any haplotype in this cluster is removed from any other by no more than 20 bp steps, while the cluster as a whole has a distance of >100 bp from the sister species *E. felidis*. The use of *E. granulosus (s.s.)* as a name for this cluster would therefore appear to be the most reasonable approach. If subdivisions of this taxon should become necessary, they will have to be based on convincing additional information concerning biological characteristics (host affinities, morphology) and nuclear sequence data.

Globally, the principal intermediate hosts for this taxon are sheep, although fertile infections have been recorded from a wide range of livestock and herbivorous wildlife species worldwide,



including equids (Cardona and Carmena, 2013; Thompson and McManus, 2001). Cattle are frequently infected with this taxon in many parts of the world, but they seem to contribute little to the transmission as the majority of cysts do not reach fertility (McManus and Thompson, 2003). *E. granulosus* s.s. is the most frequent agent of human cystic echinococcosis worldwide: 88% of 1661 genotyped human isolates belonged to this species (Alvarez Rojas et al., 2014). Exceptions are only countries where *E. granulosus* s.s. is absent or rare in animals in favour of other *Echinococcus* spp., as is the case in Sudan and Egypt (Khalifa et al., 2014; Omer et al., 2011). Cysts are often fertile in humans, and numerous observations indicate that the high number of cases may be due to increased infectivity (or pathogenicity) of *E. granulosus* s.s. compared to other *Echinococcus* species. Although the typical lifecycle patterns involve livestock and domestic dogs, *E. granulosus* s.s. is also known from wild carnivores in eastern Europe and (probably) Iran (Beiromvand et al., 2011; Breyer et al., 2004) and from wild sheep in Turkey (Simsek and Eroksuz, 2009), possibly as a spill-over from domestic transmission. Whether the latter also holds true for recent records in lions, spotted hyenas and wildebeests in conservation areas of Kenya, remains to be confirmed (Kagendo et al., 2014). In any case, there is now a surprising number of records from wild carnivores of eastern Africa. These may not only contribute to the lifecycle in the livestock-wildlife interface near conservation areas, but also as scavengers of livestock carcasses in areas that are now depleted of large wild herbivores, e.g. golden and black-backed jackals in northwestern Kenya (Macpherson and Wachira, 1997). Regional differences in haplotype diversity led to a hypothesis on the origin of *E. granulosus* s.s. in a wildlife cycle in western Asia and its subsequent spread to other regions in the wake of livestock domestication. Compared with a centre of high diversity in western Asia and the Middle East, the complexity of haplotype networks decreases toward Europe and eastern Asia and appears to be particularly low in South America (Casulli et al., 2012; Yanagida et al., 2012).

## 2.2. *Echinococcus felidis*

The species was originally described from lions of the Transvaal region of South Africa. Even after the synonymisations by Rausch and Nelson (1963) and Rausch (1967), it was tentatively retained as a separate taxon (as subspecies *E. g. felidis* or, later, as the 'lion strain'). This was mainly based on the suitability of a member of the cat family as host for the adult stage, which was thought at the time to be an unusual feature for *E. granulosus*. Recent molecular characterisation of material from Ugandan lions and preserved 'historical' worm specimens from South Africa showed, that this taxon belongs to the same clade as *E. granulosus* s.s., but is sufficiently distinct to deserve species status (Fig. 1) (Hüttner et al., 2008). As shown in the haplotype network of the mitochondrial *cox1* gene, it is removed from the *E. granulosus* s.s. cluster by more than 100 hypothetical base pair exchanges (Fig. 2).

By now, molecularly confirmed isolates of *E. felidis* are known from lions and spotted hyenas in Uganda, Kenya and South Africa; the only confirmed intermediate host record is from a Ugandan warthog (Hüttner et al., 2008; Hüttner and Romig, 2009; Hüttner et al., 2009). Other than this, the host range (including human pathogenicity) is unknown; it had not been found in 353 wildebeest (*Connochaetes mearnsi*) in the Masai Mara conservation area of Kenya, although it is widespread there in carnivores. Likewise, it was not present among 279 genotyped *Echinococcus* cysts recovered from livestock from the vicinity of Kenyan national parks (Addy et al., 2012). While *E. felidis* has not yet been found in any canid, the susceptibility of lions as definitive hosts is not a distinguishing feature for this taxon. In recent studies, *E. granulosus* s.s. and *E. equinus* were shown to be regular parasites in wild lion popu-

lations of eastern and southern Africa, respectively (Kagendo et al., 2014; Wassermann et al., 2015).

## 2.3. *Echinococcus equinus*

Williams and Sweatman (1963) characterised *E. granulosus* worms derived from horse cysts in Britain and suggested they represent a distinct subspecies, *E. granulosus equinus*. Subsequently, other studies confirmed biological differences between horse-derived *E. granulosus* and isolates originating from other host species (Kumaratilake et al., 1986; Smyth, 1977). Based on partial sequence of the *cox1* gene, the G4 genotype was characterised from two cyst isolates from horses (UK and Spain) and one from a donkey (Ireland) (Bowles et al., 1992). Species rank was suggested by Thompson and McManus (2002), and today it is firmly established as an independent species, *E. equinus*. It seems to be a highly specific parasite of Equidae (horses, donkeys and zebras) as intermediate hosts, although it was recently recorded from a captive lemur in the UK (Boufana et al., 2012).

As cysts in horses – which have been recorded from all over the world – may also belong to other *Echinococcus* spp. (Boufana et al., 2014; Varcasia et al., 2008), molecular confirmation is necessary. Thus, *E. equinus* has only been confirmed for the United Kingdom, Ireland, Germany, Italy, Spain, Tunisia and Egypt (Aboelhadid et al., 2013; Blutke et al., 2010; Mwambete et al., 2004; Smyth, 1977). However, its occurrence in other regions is highly likely, e.g. eastern Europe and South Africa, where undetermined *Echinococcus* cysts are known from horses, donkeys and zebras. Recently a wildlife cycle of *E. equinus* was discovered in the Etosha National Park of Namibia between lions and black-backed jackals as definitive hosts, and plains zebras as intermediate hosts (Wassermann et al., 2015); morphological observations and transmission studies suggest that this cycle may be widespread in southern Africa (Macpherson and Wachira, 1997).

## 2.4. *Echinococcus ortleppi*

The original description is based on adult worms from dogs of the Transvaal region of South Africa, which had been initially identified as *E. granulosus* (Ortlepp, 1934). Only later were they described as a new species for morphological differences (Lopez-Neyra and Soler Planas, 1943). Worms from dogs and jackals, that had been experimentally infected with cysts originating from cattle of the Transvaal, were later allocated to the same taxon, now named *E. granulosus ortleppi* (Verster, 1965). Later known as the cattle strain of *E. granulosus*, it was shown that it differed from other taxa in a number of characters, e.g. by cysts typically reaching fertility in cattle, morphological details of the adult worms, and a particularly short development time in dogs (Thompson et al., 1984). Apart from South Africa, where it was believed to be frequent in the cattle-raising region of Transvaal (Verster, 1965), it was later reported from Switzerland and Germany (Thompson et al., 1984; Worbes et al., 1989). Bowles et al. (1992) characterised the partial *cox1* sequence from a bovine from the Netherlands, and designated it as the G5 genotype. Under the resurrected name *E. ortleppi* (Thompson and McManus, 2002), it is now considered as a separate species, belonging to the same clade as *E. canadensis* (Fig. 1) (Nakao et al., 2013b).

*E. ortleppi* seems to be well adapted to cattle as intermediate hosts, although it can also reach fertility in other species. Fertile cysts in cattle from central European countries were common as recently as the 1980s (Hahn et al., 1986), but the parasite is now either extinct there or occurs only sporadically (Romig et al., 2006). Elsewhere, it seems to be widespread, but usually rare (Cardona and Carmena, 2013). A small number of infections have been molecularly identified from cattle in Sudan (Dinkel et al., 2004; Omer

et al., 2010), Kenya (Mbaya et al., 2014), South Africa (Mogoye et al., 2013), Brazil (de la Rue et al., 2006), Italy (Busi et al., 2007) and most recently in France (Grenouillet et al., 2014), cattle and buffaloes in India (Zhang et al., 1999), goats and sheep in Kenya (Mbaya et al., 2014), and pigs in Kenya and India (Dinkel et al., 2004; Pednekar et al., 2009). A case of monkey infection with *E. ortleppi* has been reported from Vietnam (Plesker et al., 2009), and a captive deer imported from France into the UK was found infected (Boufana et al., 2012). Only seven human cases of *E. ortleppi* infection are known from various parts of the world (Argentina, Brazil, India, Mexico, Netherlands and South Africa) (Alvarez Rojas et al., 2014). Given the ubiquitous presence of cattle and dogs as suitable hosts, the rarity of this parasite is perplexing. It may be explained by the fact, that, even in traditional pastoral societies, cattle are mostly sold alive and slaughtered in distant locations, so transmission to the local dog population is inhibited (Addy et al., 2012).

## 2.5. *Echinococcus canadensis*

As defined here, the species includes the pig, camel and cervid strains. Adult worms derived from cysts of camel and pig origin were shown to differ in various morphological characters from those of other strains, and show similarity to each other (Eckert et al., 1989; Eckert et al., 1993; Lymbery et al., 2015). This supported propositions of a specific camel strain (for the Middle East and eastern Africa), and of a pig strain (for Eastern Europe and Mexico). In addition, based on epidemiological and phenotypic features, the 'northern biotype' of *E. granulosus*, transmitted in a reindeer/moose–wolf/dog cycle, was tentatively allocated to a cervid strain (Thompson et al., 1995). The evidence for uniting pig, camel and cervid strains in a single species derived from molecular sequence data. Cysts from African camels and goats, Polish pigs and North American moose were molecularly characterised as genotypes G6, G7 and G8 (Bowles et al., 1992, 1994; Bowles and McManus, 1993b) which was later followed by genotype G9 from a human patient of Poland (Scott et al., 1997), and G10 for the 'Fennoscandian' cervid strain (Lavikainen et al., 2003). While G9, which had been based on ITS1-RFLP patterns, is now thought to represent a microvariant of G7, the other four genotypes were shown in various phylogenetic studies to form a closely related clade, with *E. ortleppi* as a sister taxon (Fig. 1) (Bowles et al., 1995; Lavikainen et al., 2006, 2003; Lymbery et al., 2015). Following a comparison of complete mitochondrial genomes, the unification of these strains as *E. canadensis* was proposed (Nakao et al., 2007). The name derived from the subspecies *E. granulosus canadensis* Webster and Cameron, 1961 described from reindeer and dogs in Canada. The name had been tentatively retained by Rausch (1967) as an alternative name for the 'northern biotype' of *E. granulosus*, while the only other scientific name that can be clearly linked to any of these strains, *E. g. borealis* Sweatman and Williams, 1963 was considered a junior synonym. Differences in host range and geography between the sylvatic (or semi-domestic) cervid strains (G8/G10) and the domestic camel and pig strains (G6/G7) had led to the proposal to regard the latter as a separate species, and the name *E. intermedium* has been suggested (Sharma et al., 2013; Thompson, 2008). However, the morphological data (and the illustrations) from the original description of *E. intermedium* (based on two worms of a Spanish dog – Lopez-Neyra and Soler Planas, 1943) do not fully agree with descriptions of worms belonging to the camel and pig strains (Eckert et al., 1989; Eckert et al., 1993). The ecological and geographic distinction between the 'domestic' G6/7 and the 'sylvatic' G8 and G10 has become blurred since G6 was recently found in wolves and reindeer in the Altai region and Yakutia, Russia (Konyayev et al., 2013). In addition, considering the existing data on mitochondrial sequences, G10 is much more closely related to G6/7 than to G8, which makes a split into *E. intermedium* (G6, G7)

vs. *E. canadensis* (G8, G10) taxonomically impossible, unless contradicting results will become available from relevant parts of the nuclear genome (Lavikainen et al., 2006; Moks et al., 2008; Nakao et al., 2013b). Most recently, a valid case was made to resolve this cluster into three species: *E. intermedium* (G6/7), *E. borealis* (G8) and *E. canadensis* (G 10) (Lymbery et al., 2015). This split is consistent with the mitochondrial phylogeny, and may in future lead to a more stable nomenclature. Yet, from a more conservative point of view, there is still a number of open issues. They mainly concern the maintenance of the proposed species' genetic identity in sympatric situations, which is crucial when independent evolutionary fates of the three lineages are proposed. To confirm this, the existing (mainly mitochondrial) data appear inadequate, and more data on nuclear gene loci will be needed from a significant number of geographically spaced isolates, in addition to more comprehensive information on biological and morphological features (Nakao et al., 2013a; Nakao et al., 2013b). Molecular distinction between G6 and G7 rests on minor differences. The pairwise divergence value of two mitochondrial genes (>2600 bp) was much lower between G6 and G7 isolates than between geographical variants of *E. multilocularis*, which makes it doubtful to address these two strains as separate entities. As some isolates cannot be clearly allocated to either of them, members of this cluster have been referred to as G6/7 (Mogoye et al., 2013; Nakao et al., 2013a; Nakao et al., 2013b). Still, biologically relevant variants may exist within the G6/7 cluster: in the Neuquén province of Argentina goats were found infected with G6, while pigs were infected with G7 (Soriano et al., 2010). This may or may not be significant (e.g., G7 is common in goats in Greece – Varcasia et al., 2007), but in any case there is a need to investigate the intraspecific genetic diversity of *E. canadensis* (including nuclear genes) and link it to epidemiologically relevant data. In the meantime, not to lose valuable information, it is important to maintain the provisional subdivision of *E. canadensis* into the four genotypes when conducting molecular surveys or isolate identification (Nakao et al., 2013a).

It had been suggested that the genotypes of *E. canadensis* are of minor relevance for human health, because case numbers are usually low in regions where these taxa predominate (e.g. Eastern Europe, Sudan/Egypt, northern parts of Eurasia and North America) and some case reports indicated a benign course of disease (Wilson et al., 1968). Also, in areas of East Africa, where both *E. canadensis* (G6) and *E. granulosus* s.s. are frequent in animals, only a small proportion of patients were found infected with the former species (Romig et al., 2011); a similar situation seems to prevail in North Africa and the Middle East (Alvarez Rojas et al., 2014). Yet, the contribution to global disease load is not negligible, as in a worldwide conspectus of 1661 human cases, 184 (11.07%) were caused by G6 or G7 (only two cases by G8 or G10). In some countries *E. canadensis* is even the predominant cause of human cystic echinococcosis, like Sudan, Egypt, Poland and Austria (Aaty et al., 2012; Omer et al., 2010; Pawlowski and Stefaniak, 2003; Schneider et al., 2008, 2010); it is important to note, however, that in these countries *E. granulosus* s.s. is absent or rare also in animals. Human cystic echinococcosis in North America of undetermined origin (G8 or G10?) was characterised by a benign course of disease (Wilson et al., 1968) and cysts of *E. canadensis* G7 were found to be smaller and more frequently asymptomatic compared to those caused by *E. granulosus* s.s. (Schneider et al., 2010). However, aggressive disease was reported from a case with confirmed G8 infection (McManus et al., 2002), and recently an affinity of *E. canadensis* G6 for the brain was suggested (Sadjjadi et al., 2013). Geographically, the G6/7 cluster is spread worldwide with the exception of Australia, eastern Asia and North America; the numerous records of molecularly characterised isolates in livestock have been recently reviewed (Cardona and Carmena, 2013). In general, pigs (including wild boar) and camelids are suitable hosts and frequently contain fertile cysts.

In the absence of pigs and camels, goats may also perpetuate the lifecycle, while sheep and cattle are rarely infected and cysts are often sterile. Cases of wildlife involvement are known from Europe (wolf, wild boar) and Siberia (wolf, reindeer) (Daniel Mwambete et al., 2004; Dinkel et al., 2004; Guerra et al., 2013; Konyaev et al., 2013; Umhang et al., 2014). Both 'cervid strains' (G8 and G10) are sympatrically distributed in the temperate to arctic regions of the northern hemisphere. Reports on possible differences in host spectrum or other biologically relevant features between these genotypes are inconclusive due to paucity of data. Both G8 and G10 have been reported from wolf–moose lifecycles, with wapiti as an additional host for G8 in North America. G10 has also been found in (semi-) domesticated reindeer, and this strain is possibly responsible for the frequent cases in reindeer and humans, which had formerly been reported from northern Fennoscandia (Lavikainen et al., 2006; Moks et al., 2008; Schurer et al., 2013; Thompson et al., 2006; Oksanen and Lavikainen, 2015).

### 3. Conclusion

The current taxonomic concept for *Echinococcus* results from critical evaluation of the biological and molecular data, which are available at this time. It provides an appropriate basis for further research by recognising distantly related taxa (cryptic species) of the *E. granulosus* (sensu lato) assemblage as individual species and by uniting closely related strains and genotypes under common species names. However, a number of taxonomic issues are still unresolved and require additional data. This concerns the correlation of molecular markers with morphological features (e.g. as a possible alternative identification method). A large body of – mostly older – literature data on morphological features of adult worms is available, but none of the descriptions (with the exception of *E. felidis*) can be conclusively correlated to genetic markers which are now used for species identification. This is mainly due to the lack of type material, storage of original material in formalin with subsequent DNA degradation and prohibitive efforts, which are now required to conduct transmission experiments. Still, correlative data between morphology and gene sequences are a matter of taxonomic priority and would make a large number of historical data accessible for epidemiological reappraisal. In addition, the extent and phylogenetic relevance of interbreeding among species is unclear, because most recent studies focused on mitochondrial sequences, which are not subject to recombination. Differences between the phylogenies of nuclear and mitochondrial genomes (e.g. by the replacement of nuclear genes due to male introgression) could explain biological differences between isolates of the same or closely related mitochondrial genotypes. In addition, the extent of intraspecific diversity is insufficiently known, particularly within *E. canadensis*. Despite this, the increased awareness for the diversity of the agents of cystic echinococcosis – now emphasised by species names rather than genotype numbers – has already led to an impressive number of recent studies on geographical spread, host range and impact on human health of the various species. It is already apparent, that they are distinct from each other to an extent that is not only biologically interesting, but also highly relevant for medical and veterinary practitioners. This includes differences in host specificity, but also possibly diverging parameters of diagnostic tests, different responses to vaccines and, importantly, possibly required modifications of clinical management due to differences in pathogenicity or response to drugs. Unfortunately, even though this variability has been recognised for a long time, a large number of epidemiological and clinical data (in particular from the largest part of the 20th century) have been published summarily under the name '*E. granulosus*'. Unless voucher specimens have been kept, a large part of this information can now not be interpreted with

certainty and is lost for retrospective analysis. In this light it is important to remind veterinarians, members of the medical professions and biologists to apply appropriate and specific diagnostic procedures when conducting studies on cystic echinococcosis, so that the causative organisms are clearly defined and the data can contribute to gradually close our gaps of knowledge.

### Acknowledgements

We thank Mar Siles-Lucas (Salamanca) for her help with literature, and we gratefully acknowledge financial support through the CESSARi network (Cystic Echinococcosis in sub-Saharan Africa Research Initiative), funded by the Deutsche Forschungsgemeinschaft (RO 3753/1-1, 2-1).

### References

- Aaty, H.E., Abdel-Hameed, D.M., Alam-Eldin, Y.H., El-Shennawy, S.F., Aminou, H.A., Makled, S.S., Darweesh, S.K., 2012. Molecular genotyping of *Echinococcus granulosus* in animal and human isolates from Egypt. *Acta Trop.* 121, 125–128.
- Aboelhadid, S.M., El-Dakhly, K.M., Yanai, T., Fukushi, H., Hassanin, K.M., 2013. Molecular characterization of *Echinococcus granulosus* in Egyptian donkeys. *Vet. Parasitol.* 193, 292–296.
- Abuladze, K.I., 1964. TAENIATA of animals and man and diseases cause by them. In: Skrjabin, A.K.I. (Ed.), *Essentials of Cestodology*. Volume IV. srael Program for Scientific Translations, 1970, Jerusalem.
- 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.
- Alvarez Rojas, C., Romig, T., Lightowlers, M.W., 2014. *Echinococcus granulosus* sensu lato genotypes infecting humans—review of current knowledge. *Int. J. Parasitol.* 44, 9–18.
- Beirovand, M., Akhlaghi, L., Fattahi Massom, S.H., Mobedi, I., Meamar, A.R., Oormazdi, H., Motevalian, A., Razmjou, E., 2011. Detection of *Echinococcus multilocularis* in carnivores in Razavi Khorasan province, Iran using mitochondrial DNA. *PLoS Negl. Trop. Dis.* 5, e1379.
- Beveridge, I., 1974. *Aspects of the taxonomy of the genus Taenia L. 1758*. University of Melbourne, Ph D Thesis.
- Blood, B.D., Lelijveld, J.L., 1969. Studies on sylvatic echinococcosis in southern South America. *Z. Tropenmed. Parasite* 20, 475–482.
- Blutke, A., Hamel, D., Hüttner, M., Gehlen, H., Romig, T., Pfister, K., Hermanns, W., 2010. Cystic echinococcosis due to *Echinococcus equinus* in a horse from southern Germany. *J. Vet. Diagn. Invest.* 22, 458–462.
- Boufana, B., Lahmar, S., Rebai, W., Ben Safta, Z., 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.
- Boufana, B., Stidworthy, M.F., Bell, S., Chantrey, J., Masters, N., Unwin, S., Wood, R., Lawrence, R.P., Potter, A., McGarry, J., Redrobe, S., Killick, R., Foster, A.P., Mitchell, S., Greenwood, A.G., Sako, Y., Nakao, M., Ito, A., Wyatt, K., Lord, B., Craig, P.S., 2012. *Echinococcus* and *Taenia* spp. from captive mammals in the United Kingdom. *Vet. Parasitol.* 190, 95–103.
- 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.P., 1994. Molecular genetic characterization of the cervid strain ('northern form') of *Echinococcus granulosus*. *Parasitology* 109, 215–221.
- Bowles, J., Blair, D., McManus, D.P., 1995. A molecular phylogeny of the genus *Echinococcus*. *Parasitology* 110 (Pt 3), 317–328.
- Bowles, J., McManus, D.P., 1993a. Molecular variation in *Echinococcus*. *Acta Trop.* 53, 291–305.
- Bowles, J., McManus, D.P., 1993b. NADH dehydrogenase 1 gene sequences compared for species and strains of the genus *Echinococcus*. *Int. J. Parasitol.* 23, 969–972.
- 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.
- Brumpt, E., Joyeux, C., 1924. Description d'un nouvel echinoque: *Echinococcus cruzi* n. sp. *Ann. Parasitol. Hum. Comp.* 3, 226–231.
- Busi, M., Snabel, 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.
- Cameron, T.W.M., 1926. Observation on the genus *Echinococcus* Rudolphi, 1801. *IV. J. Helminthol.*, 13–22.
- Cardona, G.A., Carmona, D., 2013. A review of the global prevalence, molecular epidemiology and economics of cystic echinococcosis in production animals. *Vet. Parasitol.* 192, 10–32.



- 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.
- Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* 9, 1657–1659.
- Daniel Mwambete, K., Ponce-Gordo, F., Cuesta-Bandera, C., 2004. Genetic identification and host range of the Spanish strains of *Echinococcus granulosus*. *Acta Trop.* 91, 87–93.
- de la Rue, M.L., Dinkel, A., Mackenstedt, U., Romig, T., 2006. New data on *Echinococcus* spp. in Southern Brazil. *Rev. Inst. Med. Trop. Sao Paulo* 48, 103–104.
- Diesing, K.M., 1863. Revision der Cephalocotyteen. Abteilung: Paramerocotylen. Sitzungsber. K. Akad. Wissensch. Wien Math-Naturw. 48.
- Dinkel, A., Njoroge, E.M., Zimmermann, A., Wälz, M., Zeyhle, E., Elmahdi, I.E., Mackenstedt, U., Romig, T., 2004. A PCR system for detection of species and genotypes of the *Echinococcus granulosus*-complex, with reference to the epidemiological situation in eastern Africa. *Int. J. Parasitol.* 34, 645–653.
- Eckert, J., Thompson, R.C., Lymbery, A.J., Pawlowski, Z.S., Gottstein, B., Morgan, U.M., 1993. Further evidence for the occurrence of a distinct strain of *Echinococcus granulosus* in European pigs. *Parasitol. Res.* 79, 42–48.
- Eckert, J., Thompson, R.C., Michael, S.A., Kumaratilake, L.M., el-Sawah, H.M., 1989. *Echinococcus granulosus* of camel origin: development in dogs and parasite morphology. *Parasitol. Res.* 75, 536–544.
- Grenouillet, F., Umhang, G., Arbez-Gindre, F., Manton, G., Delabrousse, E., Millon, L., Boué, F., 2014 Dec. *Echinococcus ortleppi* infections in humans and cattle, France. *Emerg. Infect. Dis.* 20, in press.
- Guerra, D., Armua-Fernandez, M.T., Silva, M., Bravo, I., Santos, N., Deplazes, P., Madeira de Carvalho, L.M., 2013. Taeniid species of the Iberian wolf (*Canis lupus signatus*) in Portugal with special focus on *Echinococcus* spp. *Int. J. Parasitol. Parasites Wildl.* 2, 50–53.
- Hahn, E., Kuske, V., Hörchner, F., Reuter, G., 1986. Zur Verbreitung der zystischen Echinokokkose beim Rind in Deutschland. *Dtsch. Tierärztl. Wochenschr.* 93, 445–447.
- Hüttner, M., Nakao, M., Wassermann, T., Siefert, L., Boomker, J.D., Dinkel, A., Sako, Y., Mackenstedt, U., Romig, T., Ito, A., 2008. Genetic characterization and phylogenetic position of *Echinococcus felidis* (Cestoda: Taeniidae) from the African lion. *Int. J. Parasitol.* 38, 861–868.
- Hüttner, M., Romig, T., 2009. *Echinococcus* species in African wildlife. *Parasitology* 136, 1089–1095.
- Hüttner, M., Siefert, L., Mackenstedt, U., Romig, T., 2009. A survey of *Echinococcus* species in wild carnivores and livestock in East Africa. *Int. J. Parasitol.* 39, 1269–1276.
- Kagendo, D., Magambo, J.K., Agola, E.L., Njenga, S.M., Zeyhle, E., Kakundi, E.M., Njeru, P.G., Mbae, C., Muchiri, E.M., Wassermann, M., Kern, P., Romig, T., 2014. A survey for *Echinococcus* spp. of carnivores in six wildlife conservation areas in Kenya. *Parasitol. Int.* 63, 604–611.
- Khalifa, N.O., Khater, H.F., Fahmy, H.A., Radwan, M.E.I., Afify, J.S.A., 2014. Genotyping and phylogenetic analysis of cystic echinococcosis isolated from camels and humans in Egypt. *Am. J. Epidemiol. Inf. Dis.* 2, 74–82.
- Konyaev, S.V., Yanagida, T., Nakao, M., Ingovatova, G.M., Shoykhet, Y.N., Bondarev, A.Y., Odnokurtsev, V.A., Loskutova, K.S., Lukmanova, G.I., Dokuchaev, N.E., Spiridonov, S., Alshinecky, M.V., Sivkova, T.N., Andreyanov, O.N., Abramov, S.A., Krivopalov, A.V., Karpenko, S.V., Lopatina, N.V., Dupal, T.A., Sako, Y., Ito, A., 2013. Genetic diversity of *Echinococcus* spp. in Russia. *Parasitology* 140, 1637–1647.
- Kumaratilake, L.M., Thompson, R.C., 1982. A review of the taxonomy and speciation of the genus *Echinococcus* Rudolphi 1801. *Z. Parasitenkd.* 68, 121–146.
- Kumaratilake, L.M., Thompson, R.C., Eckert, J., 1986. *Echinococcus granulosus* of equine origin from different countries possess uniform morphological characteristics. *Int. J. Parasitol.* 16, 529–540.
- Lavikainen, A., Lehtinen, M.J., Laaksonen, S., Agren, E., Oksanen, A., Meri, S., 2006. Molecular characterization of *Echinococcus* isolates of cervid origin from Finland and Sweden. *Parasitology* 133, 565–570.
- Lavikainen, A., Lehtinen, M.J., Meri, T., Hirvela-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.
- Lopez-Neyra, C.R., Soler Planas, M.A., 1943. Revision del genero *Echinococcus* Rud y description de una especie nueva Parasita intestinal del porro en Almeria. *Rev. Iber. Parasitol.* 3, 169–194.
- 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.
- Macpherson, C.N.L., Wachira, T.M., 1997. Cystic echinococcosis in Africa south of the Sahara. In: Andersen, F.L., Ouhelli, H., Kachani, M. (Eds.), *Compendium of Cystic Echinococcosis in Africa and in Middle Eastern Countries with Special Reference to Morocco*. Brigham Young University, Provo Utah, pp. 245–277.
- Mbaya, H., Magambo, J., Njenga, S., Zeyhle, E., Mbae, C., Mulinge, E., Wassermann, M., Kern, P., Romig, T., 2014. *Echinococcus* spp. in central Kenya: a different story. *Parasitol. Res.* 113, 3789–3794.
- McManus, D.P., Thompson, R.C., 2003. Molecular epidemiology of cystic echinococcosis. *Parasitology* 127 (Suppl.), S37–S51.
- McManus, D.P., Zhang, L., Castrodale, L.J., Le, T.H., Pearson, M., Blair, D., 2002. Short report: molecular genetic characterization of an unusually severe case of hydatid disease in Alaska caused by the cervid strain of *Echinococcus granulosus*. *Am. J. Trop. Med. Hyg.* 67, 296–298.
- Mogoye, B.K., Menezes, C.N., Wong, M.L., Stacey, S., von Delft, D., Wahlers, K., Wassermann, M., Romig, T., Kern, P., Grobusch, M.P., Frean, J., 2013. First insights into species and genotypes of *Echinococcus* in South Africa. *Vet. Parasitol.* 196, 427–432.
- Moks, E., Jogisalu, 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.
- Mwambete, D.K., Ponce-Gordo, F., Cuesta-Bandera, C., 2004. Genetic identification and host range of the Spanish strains of *Echinococcus granulosus*. *Acta Trop.* 91, 87–93.
- Nakao, M., Lavikainen, A., Yanagida, T., Ito, A., 2013a. Phylogenetic systematics of the genus *Echinococcus* (Cestoda: Taeniidae). *Int. J. Parasitol.* 43, 1017–1029.
- Nakao, M., McManus, D.P., Schantz, P.M., Craig, P.S., Ito, A., 2007. A molecular phylogeny of the genus *Echinococcus* inferred from complete mitochondrial genomes. *Parasitology* 134, 713–722.
- Nakao, M., Yanagida, T., Konyaev, S., Lavikainen, A., Odnokurtsev, V.A., Zaikov, V.A., Ito, A., 2013b. Mitochondrial phylogeny of the genus *Echinococcus* (Cestoda: Taeniidae) with emphasis on relationships among *Echinococcus canadensis* genotypes. *Parasitology* 140, 1625–1636.
- Omer, R.A., Dausgchies, A., Romig, T., 2011. Cystic echinococcosis in Sudan and South Sudan: research history of a neglected zoonosis. *Berl. Munch. Tierärztl. Wochenschr.* 124, 521–527.
- Omer, R.A., Dinkel, A., Romig, T., Mackenstedt, U., Elnahas, A.A., Aradaib, I.E., Ahmed, M.E., Elmaliik, K.H., Adam, A., 2010. A molecular survey of cystic echinococcosis in Sudan. *Vet. Parasitol.* 169, 340–346.
- Ortlepp, J.R., 1934. *Echinococcus* in dogs from Pretoria and vicinity. *Onderstepoort J. Vet. Sci. Anim. Ind.* 3, 97–108.
- Ortlepp, J.R., 1937. South African Helminths—Part I. *Onderstepoort J. Vet. Sci. Anim. Ind.* 9, 311–336.
- Oksanen, A., Lavikainen, A., 2015. Host ecology and *Echinococcus canadensis* transmission in the North. *Vet. Parasitol.*
- Pawlowski, Z., Stefaniak, J., 2003. The pig strain of *Echinococcus granulosus* in humans: a neglected issue. *Trends Parasitol.* 19, 439.
- Pednekar, R.P., Gatne, M.L., Thompson, R.C., Traub, R.J., 2009. Molecular and morphological characterisation of *Echinococcus* from food producing animals in India. *Vet. Parasitol.* 165, 58–65.
- Plesker, R., Nadler, T., Dinkel, A., Romig, T., 2009. A case of an *Echinococcus ortleppi* infestation in a red-shanked douc langur (*Pygathrix nemaeus*) in northern Vietnam. *Vietnamese J. Primatol.* 3, 75–81.
- Rausch, R.L., 1967. A consideration of infraspecific categories in the genus *Echinococcus* Rudolphi, 1801 (Cestoda: Taeniidae). *J. Parasitol.* 53, 484–491.
- Rausch, R.L., Bernstein, J.J., 1972. *Echinococcus vogeli* spp. n. (Cestoda: Taeniidae) from the bush dog, *Speothos venaticus* (Lund.). *Z. Tropenmed. Parasite* 23, 25–34.
- Rausch, R.L., Nelson, G.S., 1963. A review of the genus *Echinococcus* Rudolphi 1801. *Ann. Trop. Med. Parasitol.* 57, 127–135.
- Rausch, R.L., Schiller, E.L., 1954. Studies on the helminth fauna of Alaska. 24. *Echinococcus sibiricensis* n. sp. from St. Lawrence Island. *J. Parasitol.* 40, 659–662.
- Romig, T., Dinkel, A., Mackenstedt, U., 2006. The present situation of echinococcosis in Europe. *Parasitol. Int.* 55 (Suppl.), S187–S191.
- Romig, T., Omer, R.A., Zeyhle, E., Hüttner, M., Dinkel, A., Siefert, L., Elmahdi, I.E., Magambo, J., Ocaido, M., Menezes, C.N., Ahmed, M.E., Mbae, C., Grobusch, M.P., Kern, P., 2011. Echinococcosis in sub-Saharan Africa: emerging complexity. *Vet. Parasitol.* 181, 43–47.
- Sadjjadi, S.M., Mikaeili, F., Karamian, M., Maraghi, S., Sadjjadi, F.S., Shariat-Torbaghan, S., Kia, E.B., 2013. Evidence that the *Echinococcus granulosus* G6 genotype has an affinity for the brain in humans. *Int. J. Parasitol.* 43, 875–877.
- Schantz, P.M., Colli, C., Cruz-Reyes, A., Prezioso, U., 1976. Sylvatic echinococcosis in Argentina. 2. Susceptibility of wild carnivores to *Echinococcus granulosus* (Batsch 1786) and host-induced morphological variation. *Tropenmed. Parasitol.* 27, 70–78.
- Schneider, R., Gollackner, B., Edel, B., Schmid, K., Wrba, F., Tucek, G., Walochnik, J., Auer, H., 2008. Development of a new PCR protocol for the detection of species and genotypes (strains) of *Echinococcus* in formalin-fixed, paraffin-embedded tissues. *Int. J. Parasitol.* 38, 1065–1071.
- Schneider, R., Gollackner, B., Schindl, M., Tucek, G., Auer, H., 2010. *Echinococcus canadensis* G7 (pig strain): an underestimated cause of cystic echinococcosis in Austria. *Am. J. Trop. Med. Hyg.* 82, 871–874.
- Schurer, J., Shury, T., Leighton, F., Jenkins, E., 2013. Surveillance for *Echinococcus canadensis* genotypes in Canadian ungulates. *Int. J. Parasitol. Parasites Wildl.* 2, 97–101.
- 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 (Pt 1), 37–43.
- Sharma, M., Sehgal, R., Fomda, B.A., Malhotra, A., Malla, N., 2013. Molecular characterization of *Echinococcus granulosus* cysts in north Indian patients: identification of G1, G3, G5 and G6 genotypes. *PLoS Negl. Trop. Dis.* 7, e2262.
- Shul'ts, R.S., 1962. On the epidemiology of echinococcosis and methods of its control. *Med. Parasite* 31, 272.
- Simsek, S., Erokusuz, Y., 2009. Occurrence and molecular characterization of *Echinococcus granulosus* in Turkish mouflon (*Ovis gmelinii anatolica*). *Acta Trop.* 109, 167–169.



- Smyth, J.D., 1977. Strain differences in *Echinococcus granulosus*, with special reference to the status of equine hydatidosis in the United Kingdom. *Trans. R. Soc. Trop. Med. Hyg.* 71, 93–100.
- Snabel, V., Altintas, N., D'Amelio, S., Nakao, M., Romig, T., Yolasisgiz, A., Gunes, K., Turk, M., Busi, M., Hüttner, M., Sevcova, D., Ito, A., Dubinsky, P., 2009. Cystic echinococcosis in Turkey: genetic variability and first record of the pig strain (G7) in the country. *Parasitol. Res.* 105, 145–154.
- Soriano, S.V., Pierangeli, N.B., Pianciola, L., Mazzeo, M., Lazzarini, L.E., Saiz, M.S., Kossman, A.V., Bergagna, H.F., Chartier, K., Basualdo, J.A., 2010. Molecular characterization of *Echinococcus* isolates indicates goats as reservoir for *Echinococcus canadensis* G6 genotype in Neuquen, Patagonia Argentina. *Parasitol. Int.* 59, 626–628.
- Sweatman, G.K., Williams, R.J., 1963. Comparative studies on the biology and morphology of *Echinococcus granulosus* from domestic livestock, moose and reindeer. *Parasitology* 53, 339–390.
- Szidat, L., 1960. *Echinococcus patagonicus* sp. nov. (Cestoda) parasito del zorro *Dusicyon culpaeus culpaeus* (Mol.). *Neotropica* 6, 13–16.
- Szidat, L., 1967. *Echinococcus pampeanus*—Una nueva especie de la Argentina, parasito de *Felis colocolo pajeros* Desmarest 1916 (Cestoda). *Neotropica* 13, 90–96.
- Szidat, L., 1971. *Echinococcus cepanzeri* sp. nov. (Cestoda Taeniidae) del zorro gris, *Dusicyon gymnocercus*, de la provincia de Buenos Aires. *Neotropica* 17, 1–4.
- Tang, C., Cui, G., Qian, Y., Kang, Y., Wang, Y., Peng, W., Lu, H., Chen, D., 2007. Studies on the alveolar *Echinococcus* species in northward Daxingan mountains, Inner Mongolia, China. III. *Echinococcus russicensis* sp. nov. *Chin. J. Zoonoses* 23, 957–963.
- Tappe, D., Kern, P., Frosch, M., 2010. A hundred years of controversy about the taxonomic status of *Echinococcus* species. *Acta Trop.* 115, 167–174.
- Thompson, R.C., 2008. The taxonomy, phylogeny and transmission of *Echinococcus*. *Exp. Parasitol.* 119, 439–446.
- Thompson, R.C., Boxell, A.C., Ralston, B.J., Constantine, C.C., Hobbs, R.P., Shury, T., Olson, M.E., 2006. Molecular and morphological characterization of *Echinococcus* in cervids from North America. *Parasitology* 132, 439–447.
- Thompson, R.C., Kumaratilake, L.M., Eckert, J., 1984. Observations on *Echinococcus granulosus* of cattle origin in Switzerland. *Int. J. Parasitol.* 14, 283–291.
- Thompson, R.C., Lymbery, A.J., Constantine, C.C., 1995. Variation in *Echinococcus*: towards a taxonomic revision of the genus. *Adv. Parasitol.* 35, 145–176.
- Thompson, R.C., McManus, D., 2001. Aetiology: parasites and life-cycles. In: Eckert, J., Gemmell, M.A., Meslin, F.X., Pawowski, Z.S. (Eds.), *WHO/OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern*. World Health Organisation / World Organisation of Animal Health, Paris, pp. 1–16.
- Thompson, R.C., McManus, D.P., 2002. Towards a taxonomic revision of the genus *Echinococcus*. *Trends Parasitol.* 18, 452–457.
- Umhang, G., Richomme, C., Hormaz, V., Boucher, J.M., Boue, F., 2014. Pigs and wild boar in Corsica harbor *Echinococcus canadensis* G6/7 at levels of concern for public health and local economy. *Acta Trop.* 133, 64–68.
- 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.
- Varcasia, A., Garippa, G., Pipia, A.P., Scala, A., Brianti, E., Giannetto, S., Battelli, G., Poglajen, G., Micagni, G., 2008. Cystic echinococcosis in equids in Italy. *Parasitol. Res.* 102, 815–818.
- Verster, A.J., 1965. Review of *Echinococcus* species in South Africa. *Onderstepoort J. Vet. Res.* 32, 7–118.
- Vogel, H., 1955. Über den Entwicklungsgang und die Artzugehörigkeit des Europäischen Alveolar *Echinococcus*. *Dtsch. Med. Wochenschr.* 80, 931–932.
- Vogel, H., 1957. Über den *Echinococcus multilocularis* Süddeutschlands. I. Das Bandwurmsstadium von Stämmen menschlicher und tierischer Herkunft. *Z. Tropenmed. Parasitol.* 8, 404–454.
- 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.
- Wassermann, A., Aschenborn, O., Aschenborn, J., Mackenstedt, U., Romig, T., 2015. A sylvatic lifecycle of *Echinococcus equinus* in the Etosha National Park, Namibia. *Int. J. Parasitol. Parasites Wildl.* 4, 97–103, in press.
- Webster, G.A., Cameron, T.W.M., 1961. Observations on experimental infections with *Echinococcus* in rodents. *Can. J. Zool.* 39, 877–889.
- Williams, R.J., Sweatman, G.K., 1963. On the transmission, biology and morphology of *Echinococcus granulosus equinus*, a new subspecies of hydatid tapeworm in horses in Great Britain. *Parasitology* 53, 391–407.
- Wilson, J.F., Diddams, A.C., Rausch, R.L., 1968. Cystic hydatid disease in Alaska. A review of 101 autochthonous cases of *Echinococcus granulosus* infection. *Am. Rev. Respir. Dis.* 98, 1–15.
- Worbes, H., Thompson, R.C., Eckert, J., 1989. Occurrence of the cattle strain of *Echinococcus granulosus* in the German Democratic Republic. *Parasitol. Res.* 75, 495–497.
- Xiao, N., Qiu, J., Nakao, M., Li, T., Yang, W., Chen, X., Schantz, P.M., Craig, P.S., Ito, A., 2005. *Echinococcus shiquicus* n. sp., a taeniid cestode from Tibetan fox and plateau pika in China. *Int. J. Parasitol.* 35, 693–701.
- Xiao, N., Qiu, J., Nakao, M., Li, T., Yang, W., Chen, X., Schantz, P.M., Craig, P.S., Ito, A., 2006. *Echinococcus shiquicus*, a new species from the Qinghai-Tibet plateau region of China: discovery and epidemiological implications. *Parasitol. Int.* 55 (Suppl.), S233–S236.
- 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.
- Zhang, L., Gasser, R.B., Zhu, X., McManus, D.P., 1999. Screening for different genotypes of *Echinococcus granulosus* within China and Argentina by single-strand conformation polymorphism (SSCP) analysis. *Trans. R. Soc. Trop. Med. Hyg.* 93, 329–334.