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Review

Mutation scanning analysis of genetic variation within and among *Echinococcus* species – implications and future prospects

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Abbreviations: **AE**, Alveolar echinococcosis; **AgB**, Antigen B; **CE**, Cystic echinococcosis; **HRM**, high-resolution melting-curve analysis; **ITS**, internal transcribed spacers of nuclear ribosomal DNA; **MEE**, Multilocus enzyme electrophoresis; **PE**, Polycystic echinococcosis; **RAPD**, Random amplification of polymorphic DNA; **RFLP**, restriction fragment length polymorphism; **SSCP**, single-strand conformation polymorphism

(ABSTRACT)

Adult tapeworms of the genus *Echinococcus* (family Taeniidae) occur in the small intestines of carnivorous definitive hosts and are transmitted to particular intermediate mammalian hosts, in which they develop as fluid-filled larvae (cysts) in internal organs (usually lung and liver), causing the disease echinococcosis. *Echinococcus* species are of major medical importance and also cause losses to the meat and livestock industries, mainly due to the condemnation of infected offal. Decisions regarding the treatment and control of echinococcosis rely on the accurate identification of species and population variants (strains). Conventional, phenetic methods for specific identification have some significant limitations. Despite advances in the development of molecular tools, there has been limited application of mutation scanning methods to species of *Echinococcus*. Here, we briefly review key genetic markers used for the identification of *Echinococcus* species and techniques for the analysis of genetic variation within and among populations, and the diagnosis of echinococcosis. We also discuss the benefits of utilizing mutation scanning approaches to elucidate the population genetics and epidemiology of *Echinococcus* species. These benefits are likely to become more evident following the complete characterisation of the genomes of *E. granulosus* and *E. multilocularis*.

Keywords:

Echinococcus / Epidemiology / Mutation scanning / Ecology / Taxonomy / Specific and genotypic identification

1 Introduction

Echinococcosis is a zoonotic infection or disease caused by tapeworms of the genus *Echinococcus* (Platyhelminthes: Eucestoda: Taeniidae). The life cycles of these parasites involve two mammalian hosts. The adult worms inhabit the small intestine of carnivorous definitive hosts (usually canids or felids) and produce eggs containing infective larvae (=oncospheres) (Table 1). Following the ingestion of eggs by an intermediate host (a range of domestic and wild animals as well as humans), a larval (or metacestode) stage develops, usually over many years, in internal organs, leading to echinococcosis. There are three main forms of this disease. Cystic echinococcosis (CE), caused by *E. granulosus*, is the most common form of human disease and is estimated to account for > 95% of the 2-3 million cases worldwide, while CE of livestock animals results in losses to the meat industry due to the condemnation of infected offal (mainly lungs and livers) [1-5]. Alveolar echinococcosis (AE), caused by *E. multilocularis*, occurs in rodents (intermediate hosts) and accidentally infects humans, in which disease is fatal [1, 2, 6]. Similarly, polycystic echinococcosis (PE) of humans is also a fatal disease. PE is caused by *E. vogeli*, and mainly infects the lungs and liver, whereas echinococcosis caused by *E. oligarthrus* is limited to the heart and eyes [2, 7].

The accurate diagnosis of *Echinococcus* infections in both definitive and intermediate hosts is central to investigating the epidemiology of echinococcosis, and underpins treatment and control programs. Traditionally, the identification and characterization of *Echinococcus* species and subspecies (population variants or strains) was based on a combination of biological, epidemiological and morphological criteria, including (i) the morphology of the adult stage (length of adult worms, relative position of the mature segment, shape and size of rostellar hooks, position of genital pore along the margin, shape of the gravid uterus); (ii)

features of the cyst (single or multiple, unilocular or multilocular, predilection site, diameter and presence or absence of protoscoleces); and (iii) the extent of host specificity in different geographical regions [9, 12-15]. However, the identification and differentiation of population variants is not possible based exclusively on phenetic data.

Traditional biochemical and molecular approaches, such as multilocus enzyme electrophoresis (MEE) and restriction fragment length polymorphism (RFLP)-based Southern blot analysis, had assisted in the genetic identification and characterization of *Echinococcus* variants (strains) from different hosts [9, 14, 16-18]. Particularly methods based on the use of the polymerase chain reaction (PCR) [19, 20] have found broad applicability to population genetic and epidemiological investigations of *Echinococcus*, mainly because their sensitivity permits the analysis of nuclear or mitochondrial gene regions from minute amounts of genomic DNA from fresh, frozen or ethanol fixed parasite material [21-28]. In the present article, we review genetic markers employed for the identification of *Echinococcus* species, and tools for the analysis of genetic variation within and among species. We also discuss the advantages and disadvantages of selected techniques and emphasize the benefits of utilizing mutation scanning to elucidate the systematics, population genetics and epidemiology of members within this genus, particularly in light of the imminent genome sequences for selected *Echinococcus* species.

2 Main genetic markers and methods used, and their attributes

PCR-coupled techniques, employing primer pairs for the specific amplification of different gene regions, followed by enzymatic cleavage, or sequencing, have often been used to identify, characterize or classify species of *Echinococcus*. Currently recognized are five species of *Echinococcus* and at least 10 genotypes of *E. granulosus* (Tables 1 and 2) (reviewed by [9, 10, 26]). The key loci used are within mitochondrial (mt) genes or nuclear

Mitochondrial DNA (mtDNA) regions have been used most commonly for the identification and delineation of closely related species because of its relatively rapid rate of evolution. This DNA is haploid [31], thus simplifying sequencing and analysis. Complete or almost complete mtDNA sequences have been determined for a number of taeniid cestodes [32], and they provide a rich source of genetic markers for systematic and population genetic studies [33]. The mitochondrial genomes of the *Echinococcus* species sequenced to date (i.e. *E. granulosus*, *E. canadensis*, *E. multilocularis*, *E. oligarthus*, *E. orteppi*, *E. shiquicus* and *E. vogeli*) are similar in structure to those of *Taenia* species and/or other metazoans [26, 32-38]. They contain 12 protein-coding genes, including adenosine triphosphatase subunit 6 (*atp6*), cytochrome *c* oxidase (*cox1-cox3* subunits) complexes, and cytochrome *b* (*cob*) and nicotinamide dehydrogenase (subunits *nad1-nad6* and *nad4L*). These genes lack introns and usually abut one another, or are separated by a small number of nucleotides, and are transcribed in the same direction; some gene regions, such as *nad4* and *nad4L*, can overlap slightly. In accordance with most other parasitic worms studied to date [38, 39], *atp8* gene is absent, which contrasts the situation for helminths such as *Trichinella* and *Trichuris* species (Enoplea) [40-42] and other metazoan groups [28, 38]. Two ribosomal RNA genes, the large subunit (*rrnL*) and small subunit (*rrnS*) are present as well as 22 tRNA genes. In *Echinococcus*, there are two relatively long non-coding regions (NRs), which are likely to be associated with replication and/or transcription. The mitochondrial markers most commonly utilized for systematic and population genetic studies of cestodes are within the *cox1*, *nad1*, *cob* and rRNA genes [26, 43]. Some of these mitochondrial and/or selected nuclear markers have been employed also in diagnostic assays based on specific enzymatic amplification [44-50].

Nuclear ribosomal DNA (rDNA) also provides markers for specific and genotypic identification and characterization. Nuclear rDNA of eukaryotes, including taeniid cestodes,

represents a large multigene family, consisting of tandemly arrayed sequence repeats (often tens or hundreds), usually found in clusters in specific chromosomes [51]. Through various molecular processes, such rDNA sequences exhibit patterns of “concerted evolution”, which usually lead to sequence homogenization that is greater within a species than among species [51-57]. As a consequence of this evolution, rDNA can provide useful, specific markers for parasites. For instance, internal and external transcribed spacers (ITS and ETS, respectively) as well as the 28S rRNA gene have been demonstrated to provide useful genetic markers for taeniids and other helminths [26, 28, 58-61], applicable to PCR-based systems. Although within some species of helminths, sequence variation in ITS rDNA can be low [28], *Echinococcus* and other taeniid species tend to exhibit significant length and/or sequence heterogeneity within this region, apparently reflecting intra-isolate, intra-individual or population variation [24, 27, 62-65].

Anonymous markers, defined by random amplification of polymorphic DNA (RAPD) analysis [66, 67], have been used for the purpose of detecting genetic variation within and among populations of a particular species. These markers, displayed electrophoretically following RAPD, have been employed to infer “genetic” variation within species [68-73]. Although RAPD was originally considered a useful approach, because of its ability to amplify from small amounts of genomic DNA and its capacity to rapidly screen genome/s for variation without requiring prior DNA sequence information, there are significant problems with the specificity and reproducibility of RAPD data due to the low stringency often used in PCR [28, 74, 75]. Importantly, the display of bands on agarose or polyacrylamide gels, based on size alone, provides phenetic (rather than genetic) characters; these characters are anonymous, thus limiting the value of such datasets. However, once defined by sequencing, “sequence-characterized amplified region” (SCAR) markers [76] can be used in diagnostic assays following the design of specific oligonucleotide primers for use in PCR.

Also satellite DNA [77, 78] has been used increasingly for studies of genetic variation

in and biology of *E. granulosus* [79-82] and *E. multilocularis* [82-92]. For instance, Knapp et al. [90] described how studies of *E. multilocularis*, a species considered previously as notorious for its genetic homogeneity [93], were now possible. These authors [90] explained how rapidly evolving genetic markers, such as microsatellites, are applicable to studying the genetic diversity and structures of parasite populations. In their opinion, the multi-locus microsatellite, designated EmsB (tandemly repeated in the *E. multilocularis* genome), offers an opportunity as a “molecular tracker” of the temporal and spatial transmission of *Echinococcus* to various hosts. Despite of this optimism, some researchers might caution the use of satellite analyses in the absence of definitive genomic sequence data.

PCR-coupled restriction fragment length polymorphism (PCR-RFLP) analysis has also been used for the genetic identification and/or classification of strains of *E. granulosus*. Bowles and McManus [24] were the first authors to rapidly and reliably identify and delineate species and genotypes of *Echinococcus* by PCR-RFLP analysis of the first internal transcribed spacer (ITS-1) of nuclear rDNA. These and other authors [22, 24, 27, 63, 65, 73, 94-118] extended the use of this method, utilizing various nuclear and mitochondrial genetic markers, to address various questions regarding the taxonomy, ecology and epidemiology of *Echinococcus* species [9, 26, 37, 119-122]. In spite of its utility, because the endonuclease/s employed only recognize a small number of potentially variable sites in DNA, RFLP analysis might not detect all of the sequence variation within amplicons [28, 123]. Therefore, direct DNA sequencing, together with comparative analyses of sequence data, remains the “gold-standard” for detecting and defining genetic variation, and can be applied to single-copy or multi-copy genes (provided that there is no significant sequence/length heterogeneity among paralogues).

DNA sequence data have added major value to comparative genetic investigations, due to the availability of information for a wide range of genes in public databases, such as GenBank (<http://www.ncbi.nlm.nih.gov>). However, in some instances, there can be limitations

in the direct sequencing of some “complex” DNA regions. If there is genetic heterogeneity or polymorphism within an isolate (e.g., among pooled protoscoleces from a cyst), such as can be the case for the first (ITS-1) or second (ITS-2) ITS regions [64, 124], it may not be possible to determine an unambiguous sequence from an amplicon. Moreover, the direct sequencing of large numbers of samples might be time-consuming and costly for some parasitology laboratories and often requires bioinformatic analysis. Efficient, high-resolution analysis of large numbers of amplicons can be achieved utilizing mutation detection methods.

3 Mutation scanning-based tools for the genetic analysis of *Echinococcus*

A particularly practical electrophoretic mutation scanning approach is single-strand conformation polymorphism (SSCP) [125, 126]. This technique relies on the principle that the electrophoretic mobility of a single-stranded DNA molecule in a non-denaturing gel is highly dependent on its structure and size, offering the capacity of detecting single point mutations in PCR products of < 500 bp [126, 127]. The high mutation detection capacity and relative technical simplicity of PCR-coupled SSCP make it a powerful tool for the identification of species or genotypes, to screen for genetic variability within and among large numbers of samples, and, importantly, to screen for unknown mutations [126]. When utilized as a screening tool to assess the extent of genetic variation within a population, prior to targeted sequencing of representative samples, PCR-based SSCP decreases the cost and time associated with analyses, because of a substantial reduction (often 70-90%) in the number of samples requiring sequencing.

Various studies have employed SSCP to determine sequence variation in mitochondrial or nuclear DNA regions for the purposes of (i) classifying different genotypes of *E. granulosus* from various intermediate host species from different geographical regions, and studying their genetic and evolutionary relationships; (ii) assessing the ‘breeding system’

of *Echinococcus* species; and (iii) evaluating levels of sequence variation within a family of genes encoding Antigen B (designated AgB) (Table 3).

3.1 Classifying genotypes and studying genetic/evolutionary relationships

Some recent studies [122, 129, 130] employed mutation scanning-targeted sequencing-phylogenetic analyses to classify different genotypes of *E. granulosus* from various intermediate host species from different geographical regions (cf. Table 3). For example, Jabbar et al. [122] used non-isotopic SSCP-based analysis to genetically classify *E. granulosus* cysts from humans from Mongolia using mitochondrial DNA regions, designated *pcox-1* and *pnad-1*, within the mitochondrial cytochrome *c* oxidase subunit 1 (*cox-1*) and NADH dehydrogenase subunit 1 (*nad-1*) genes, respectively. SSCP analysis of *pcox-1* and *pnad-1* amplicons produced from genomic DNA samples from individual *E. granulosus* cysts (n = 50) from individual humans displayed four distinct electrophoretic profiles for each *pcox-1* and *pnad-1*. Direct sequencing of selected amplicons representing each of these profiles also defined four distinct sequence types for each locus, present in four different combinations (designated as haplotypes M1-M4) amongst all 50 cyst isolates. Phylogenetic analysis of concatenated sequence data for these four haplotypes, including well-defined reference sequences, inferred that 68% of cyst isolates belonged to the G1-G3 complex of *E. granulosus* (or *E. granulosus sensu stricto*), whereas the remaining (32%) were linked to the G6-G10 complex (or *E. canadensis*) (cf. Table 2). Humans infected with *E. granulosus* cysts of the G1-G3 complex originated mainly from the eastern part of Mongolia, whereas those harbouring cysts of the G6-G10 complex were linked to the western region of this country. This study provided a first glimpse of the genetic composition of *E. granulosus* from humans in Mongolia, and formed a basis for future studies of the epidemiology and ecology of the parasite(s) in animals and humans in this country.

Some investigations [122, 129, 130] emphasize the utility and effectiveness of an integrated mutation scanning-sequencing-phylogenetic analysis of mitochondrial markers for

larger scale population genetic and epidemiological studies of species and strains of *Echinococcus*. Other studies of mitochondrial genomes [26, 37, 141, 142] also underpin future analyses to establish the genetic relationships of members of the genus *Echinococcus*. In spite of significant advances [9, 10, 26, 37, 121], there is still relatively limited detailed information on the population genetic structures of *E. granulosus* and related species for many countries around the world [143, 144].

A key aspect warranting detailed, future investigation is the re-testing of hypotheses regarding evolutionary relationships of species and genotypes of *Echinococcus* (Tables 1 and 2). Phylogenetic analyses of mitochondrial gene sequence datasets [122, 129, 130] provide clear evidence that *E. felidis*, *E. multilocularis*, *E. shiquicus* and *E. vogeli* are separate species, and that *E. granulosus* is a complex of at least 10 genotypes. Specifically, genotypes G1-G10 represent *E. granulosus sensu lato*, but there is some debate as to the specific status of members within the G1-G10 complex. Currently, the proposal is that genotypes G1-G3 represent *E. granulosus sensu stricto*, and that genotypes G4, G5 and G6-G10 represent *E. equinus*, *E. orteppi* and *E. canadensis*, respectively [10, 37, 145]. Nonetheless, there is still considerable controversy [121] as to the specific status of genotype G5. Although genotype G2 was originally suggested to be distinct from genotypes G1 and G3 [21, 37], recent molecular studies [10, 122, 129, 130] indicate that G2 is embedded within the G1-G3 complex (i.e. *E. granulosus sensu stricto*). Moreover, although genotype G9 was recorded [29], its validity needs to be confirmed [146]. In addition to these issues, Saarma et al. [147] presented a novel phylogenetic tree for *Echinococcus*, based on nuclear gene sequences from published studies [11, 148]. When compared with analyses of mitochondrial gene data [37], these authors [147] observed a translocation of *E. multilocularis* and *E. shiquicus* into basal nodes in their tree. This difference in tree topology emphasizes the need to use an extensive range of both mitochondrial and nuclear markers for future phylogenetic reconstructions, preferably following large-scale mutation scanning analyses of *Echinococcus* populations.

3.2 Assessing breeding systems

Haag et al. [93, 94] employed a PCR-coupled SSCP approach to assess sequence variation in six genetic loci to evaluate the breeding systems of *E. granulosus*, *E. multilocularis* and *E. vogeli* (Table 3). In an extensive analysis of 110 larval isolates from various host species (sheep, cattle, pig and horse) and geographical regions (Australia, Europe and southern Brazil), the authors identified four genotypes of *E. granulosus*. While the genotypes infecting horses (G4) and pigs (G7) were genetically homogeneous, the genotypes in sheep (G1) and cattle (G5) displayed sequence polymorphisms in one (Ag4) and two (Ag4 and Ag6) loci, respectively. Based on these findings, the authors concluded that there was a clear genetic differentiation among genotypes, but not within genotypes, suggesting that reproduction was largely through 'self-fertilization'. Nonetheless, in some cases, mixed sequence types were detected for some nuclear loci, and the authors suggested that hybrid genotypes might occur in *E. granulosus* from cattle and sheep in southern Brazil, but not from other geographical areas [119].

3.3 Assessing sequence variation within a gene family

Mutation scanning and targeted DNA sequencing have been utilized to assess levels of sequence variation within the gene family encoding AgB of *E. granulosus* [137-139] (see Table 3). For instance, Chemale et al. [137] employed SSCP for the analysis of amplicons from 100 recombinant clones representing cDNAs and genomic DNAs encoding AgB; these authors detected different SSCP profiles among all amplicons representing the 150 amplicons and sequenced three independent clones for each profile. While no sequence heterogeneity was detected among the three independent clones representing each profile, variation of 1-35% was recorded among the sequences representing each of the three profiles. This study showed excellent mutation detection capacity of SSCP, and promise for analyses of complex gene families.

4 Prospects for semi-automated mutation scanning tools

There have been significant technical improvements to electrophoretic platforms, due to an increased demand for high throughput genetic analysis of pathogens, automation and *in silico*-handling of data. This comment applies, for instance, to different capillary electrophoretic (CE) systems [149-151]. CE-coupled SSCP has been employed as a high-throughput method for a range of applications, including the detection of single nucleotide polymorphisms (SNPs) [152-155]. This approach has not been applied widely to parasites, but could be a useful tool for the analysis of genetic variation within and among *Echinococcus* species, and offers a significant advantage in terms of its high throughput potential, given the decreased time required for analyses compared with conventional SSCP. However, a disadvantage of automated CE-based SSCP is the purchase cost (\$150,000 to \$250,000) of the required equipment. Therefore, a commercial service provider might be used; nonetheless, this might impact on the time-efficiency of the approach relative to gel electrophoresis platforms [126].

Base excision sequence scanning (BESS; ref. [156]) is another PCR-based method that can detect and/or locate DNA mutations. Although this method has been applied to *Taenia* species infecting humans [46, 157], it has not been used for the analysis of genetic variation within *Echinococcus* species. The BESS method includes two procedures that generate "T" (BESS T-Scan) and "G" ladders (BESS G-Tracker), analogous to the T and G ladders of conventional dideoxy sequencing, respectively. According to Hawkins and Hoffman [156], the procedure is relatively simple to carry out and does not require specialized equipment or optimization beyond PCR. Samples can be resolved on traditional sequencing gels or in automated sequencers, and the data produced are relatively easy to interpret, requiring a simple comparison between normal and mutant DNAs. The BESS method seems to be relatively versatile, having applications, not only for mutation detection, but also for fingerprinting and SNP discovery. However, BESS is unlikely to be applicable to DNA

regions containing sequence and length variation; also, the time and cost involved in performing this method appear to be similar to direct, automated sequencing.

There is prospect for the application of PCR-coupled high-resolution melting-curve (HRM) analysis as a mutation scanning tool, depending on nucleotide composition and size of amplicons [158]. Studies, employing different intercalating dyes, have demonstrated that complex melting profiles can be reproducible and diagnostically informative, providing a unique ‘fingerprint’ with an increased number of characters for delineating species and/or genotypes [159-162]. Programs, such as POLAND (<http://www.biophys.uni-duesseldorf.de/local/POLAND/poland.html>) and MELTSIM (<http://ftp.bioinformatics.org/pub/meltsim>), allow *in silico*-predictions of melting profiles, which can assist in establishing useful laboratory protocols for the analysis of sequences with complex melting characteristics [159]. Although PCR-coupled HRM analysis requires evaluation for gene regions for cestodes, it could offer a tool for the rapid identification or differentiation of *Echinococcus* species/genotypes in various contexts. However, although rapid to perform, PCR-based HRM analysis does not have the “preparative” qualities of non-isotopic SSCP (in that individual bands cannot be stabbed or excised from gels for subsequent sequencing), and the real-time PCR thermal cycler required for analysis is relatively costly to purchase.

PCR-coupled SSCP has proved to be practical, sensitive, specific and reproducible [126]. Provided that the method follows a standardized protocol (thus achieving a high mutation detection rate) and that results are interpreted carefully, SSCP has major analytical capacity and, together with selective sequencing and phylogenetic analyses of sequence data, is perfectly suited to investigations of the population genetics and epidemiology of *Echinococcus*, if careful consideration is given to sampling procedures and genetic markers [28, 163]. This tool also represents a platform for tackling diagnostic problems. For instance, it could be readily used to support the specific and genotypic diagnosis of echinococcosis in

humans following the surgical removal of cysts or cyst content (i.e. protoscoleces or germinal layer), which could guide decisions regarding the treatment and management of the disease.

Enhanced techniques for the isolation and specific enzymatic amplification of genetic loci from genomic DNA from tissue, faecal or environmental samples will assist biological, epidemiological and ecological studies. However, there is no universal solution to challenges associated with the isolation of minute amounts of parasite DNA from such samples and the removal of inhibitory substances, such that techniques need to be assessed and optimized in each case. Furthermore, a broad range of genetic markers (≤ 450 bp) can be utilized in SSCP in both the analytical and diagnostic settings. Depending on levels of intra- and inter-specific nucleotide variation for particular markers, pre-screening by mutation scanning can substantially decrease (often by 75-90%) the number of samples required for sequencing and the time required for bioinformatic analyses of sequence data. Importantly, SSCP analysis can display multiple sequence types within a single sample [164] and has the capacity to detect 'mixed' sequence types (e.g., in the case of nuclear markers), 'mixed' infections (consisting of multiple species or genotypes) or extraneous/erroneous contamination, which are often difficult to achieve using other genetic techniques. Therefore, in our opinion, the integrated PCR-based mutation scanning - targeted sequencing - phylogenetic approach represents a practical, 'powerful' and cost-effective tool for large-scale studies of genetic variation within *Echinococcus* at both the specific and genotypic levels, using a range of markers.

5 Concluding remarks - opportunities through large-scale high throughput sequencing of genomes

Many markers in the nuclear genomes of most taeniid cestodes still remain to be discovered. High throughput sequencing technologies, such Illumina-Solexa (www.illumina.com; [165]), provide unique opportunities for the high-throughput genomic explorations of parasites [166-

168]. Thus far, these technologies have been employed in a range of contexts, such as whole-genome sequencing, re-sequencing and transcriptomic profiling [169-174]. Although they are now having substantial impact in many biological and biomedical areas, these technologies have not yet been applied widely to parasites, largely because of the purchase, running and maintenance costs of the sequencing and computer infrastructure required, and limited research funds available in many parasitology laboratories. Nonetheless, some smaller laboratories are now undertaking transcriptomic and genomic exploration of parasites using massively parallel sequencing technology, also to underpin the direct nuclear genomic sequencing of parasitic helminths (e.g., [39, 168, 171, 172, 175]). In the future, massively parallel sequencing technologies will make it possible to directly characterize large numbers of individual specimens or isolates of *Echinococcus* from small amounts of material by their complete mitochondrial and/or nuclear genome sequences, as is currently being conducted for humans on a massive scale [169, 170, 176-178]. For instance, the long-range PCR-coupled sequencing, recently established for nematodes [38, 39, 179, 180], could be adapted to the high throughput sequencing and annotation of mitochondrial genomes of *Echinococcus* species; this technology is within reach, in terms of cost. Moreover, both mitochondrial and nuclear genomes can now be sequenced simultaneously and directly using massively parallel sequencing, such as Illumina.

Recently, a consortium of laboratories has now sequenced the genomes of *E. granulosus* (<http://www.sanger.ac.uk/resources/downloads/helminths/echinococcus-granulosus.html>) and *E. multilocularis* (<http://www.sanger.ac.uk/resources/downloads/helminths/echinococcus-multilocularis.html>), and sequence data are now accessible. However, in spite of this advance, progress on taeniids has been constrained compared with rapid advances made in many biomedical areas [169, 170, 181-184], mainly due to limitations in funding for fundamental research of tapeworms. Nonetheless, these technologies are now becoming affordable and will allow the sequencing of genomes of a wide spectrum of species and genotypes of

Echinococcus, as basis for addressing the controversies surrounding their genetic relationships. Comparative analyses of such genome sequences will also enable the rational selection of genes (possibly hundreds or thousands, displaying suitable levels of nucleotide variation within *Echinococcus* species for the particular task at hand), which could be used in population genetic studies for SNP detection by mutation scanning. Moreover, the proteins predicted from core genes (using the algorithmic pipeline GEGMA; ref. [185]) in these genomes could be compared for levels of genetic variation, and then concatenated and subjected to phylogenetic analyses to re-test hypotheses regarding the evolutionary relationships of *Echinococcus* species and genotypes. It is also expected that next-generation technologies will impact through large-scale studies of the epidemiology and ecology of *Echinococcus* species. Such fundamental studies will not only provide valuable insights into the parasites' biology, they should also elucidate patterns of disease transmission, thus supporting the prevention and control of echinococcosis.

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References

- [1] Pawłowski, Z. S., Eckert, J., Vuitton, D. A., Ammann, R. W., Kern, P., Craig, P. S., Dar, K. F., De Rosa, F., Filice, C., Gottstein, B., Grimm, F., Macpherson, C. N. L., Sato, N., Todorov, T., Uchino, J., von Sinner, W., Wen, H., in: Eckert, J., Gemmell, M. A., Meslin, F. X., Pawłowski, Z. S. (Eds.), *Manual on Echinococcus in Humans and Animals: A Public Health Problem of Global Concern*, WHO/OIE., Paris, France 2001, pp. 20–72.
- [2] Eckert, J., Deplazes, P., *Clin. Microbiol. Rev.* 2004, 17, 107–135.
- [3] Budke, C. M., Deplazes, P., Torgerson, P. R., *Emerg. Infect. Dis.* 2006, 12, 296–303.
- [4] Budke, C. M., Jiamin, Q., Qian, W., Torgerson, P. R., *Am. J. Trop. Med. Hyg.* 2005, 73, 2–10.
- [5] Wahlers, K., Menezes, C. N., Wong, M. L., Zeyhle, E., Ahmed, M. E., Ocaido, M., Stijnis, C., Romig, T., Kern, P., Grobusch, M. P., *Lancet Infect. Dis.* 2012, 12, 871–880.
- [6] Davidson, R. K., Romig, T., Jenkins, E., Tryland, M., Robertson, L. J., *Trends Parasitol.* 2012, 28, 239–247.
- [7] Rausch, R. L., D'Alessandro, A., in: Craig, P., Pawłowski, Z. (Eds.), *Cestode Zoonoses: Echinococcosis and Cysticercosis, an Emergent and Global Problem*, IOS Press Amsterdam, The Netherlands. 2002, pp. 107–113.
- [8] Jenkins, D. J., Romig, T., Thompson, R. C., *Int. J. Parasitol.* 2005, 35, 1205–1219.
- [9] Thompson, R. C., McManus, D. P., *Trends Parasitol.* 2002, 18, 452–457.
- [10] Thompson, R. C., *Exp. Parasitol.* 2008, 119, 439–446.
- [11] Xiao, N., Qiu, J., Nakao, M., Li, T., Yang, W., Chen, X., Schantz, P. M., Craig, P. S., Ito, A., *Int. J. Parasitol.* 2005, 35, 693–701.
- [12] Rausch, R. L., in: Thompson, R. C. A., Lymbery, A. J. (Eds.), *Echinococcus and hydatid disease*, CAB International, Wallingford 1995, pp. 89–134.
- [13] Lymbery, A. J., *Parasitology* 1998, 117 (Pt 2), 185–192.
- [14] Lymbery, A. J., Thompson, R. C., *Int. J. Parasitol.* 1988, 18, 803–811.
- [15] Thompson, R. C., Lymbery, A. J., *Int. J. Parasitol.* 1990, 20, 457–470.
- [16] McManus, D. P., Rishi, A. K., *Parasitology* 1989, 99 Pt 1, 17–29.
- [17] McManus, D. P., Thompson, R. C., *Parasitology* 2003, 127 Suppl, S37–51.
- [18] Hope, M., Bowles, J., McManus, D. P., *Int. J. Parasitol.* 1991, 21, 471–475.
- [19] Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G., Erlich, H., *Cold Spring Harb. Symp. Quant. Biol.* 1986, 51 Pt 1, 263–273.
- [20] Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., Erlich, H. A., *Science* 1988, 239, 487–491.
- [21] Bowles, J., Blair, D., McManus, D. P., *Mol. Biochem. Parasitol.* 1992, 54, 165–174.
- [22] Bowles, J., Blair, D., McManus, D. P., *Parasitology* 1994, 109 (Pt 2), 215–221.
- [23] Bowles, J., McManus, D. P., *Acta Trop.* 1993, 53, 291–305.
- [24] Bowles, J., McManus, D. P., *Mol. Biochem. Parasitol.* 1993, 57, 231–239.
- [25] Siles-Lucas, M., Cuesta-Bandera, C., Cesar-Benito, M., *Parasitol Res.* 1993, 79, 343–345.
- [26] McManus, D. P., *Parasitol. Int.* 2006, 55 Suppl, S31–37.
- [27] Gasser, R. B., Chilton, N. B., *Acta Trop.* 1995, 59, 31–40.
- [28] Gasser, R. B., *Vet. Parasitol.* 2006, 136, 69–89.
- [29] Scott, J. C., Stefaniak, J., Pawłowski, Z. S., McManus, D. P., *Parasitology* 1997, 114 (Pt 1), 37–43.
- [30] Lavikainen, A., Lehtinen, M. J., Meri, T., Hirvela-Koski, V., Meri, S., *Parasitology* 2003, 127, 207–215.
- [31] Avise, J. C., *Molecular Markers, Natural History and Evolution*, Chapman and Hall, New York and London 1994, pp. 511.
- [32] Le, T. H., Blair, D., McManus, D. P., *Acta Trop.* 2000, 77, 243–256.
- [33] Le, T. H., Blair, D., McManus, D. P., *Trends Parasitol.* 2002, 18, 206–213.
- [34] McManus, D. P., *Trans. R. Soc. Trop. Med. Hyg.* 2002, 96 Suppl 1, S151–157.
- [35] Nakao, M., Okamoto, M., Sako, Y., Yamasaki, H., Nakaya, K., Ito, A., *Parasitology* 2002, 124, 657–662.
- [36] Yang, Y. R., Rosenzvit, M. C., Zhang, L. H., Zhang, J. Z., McManus, D. P., *Parasitology* 2005, 131, 547–555.
- [37] Nakao, M., McManus, D. P., Schantz, P. M., Craig, P. S., Ito, A., *Parasitology* 2007, 134, 713–722.
- [38] Hu, M., Gasser, R. B., *Trends Parasitol.* 2006, 22, 78–84.
- [39] Jex, A. R., Littlewood, D. T., Gasser, R. B., *Biotechnol. Adv.* 2010, 28, 151–159.
- [40] Webb, K. M., Rosenthal, B. M., *Infect. Genet. Evol.* 2010, 10, 304–310.
- [41] Webb, K. M., Rosenthal, B. M., *Infect. Genet. Evol.* 2011, 11, 116–123.
- [42] Liu, G. H., Gasser, R. B., Su, A., Nejsun, P., Peng, L., Lin, R. Q., Li, M. W., Xu, M. J., Zhu, X. Q., *PLoS Negl. Trop. Dis.* 2012, 6, e1539.
- [43] Yamasaki, H., Nakao, M., Sako, Y., Nakaya, K., Sato, M. O., Ito, A., *Parasitol. Int.* 2006, 55 Suppl,

- S81–85.
- [44] Gottstein, B., *Clin. Microbiol. Rev.* 1992, 5, 248–261.
- [45] Siles-Lucas, M. M., Gottstein, B. B., *Trop. Med. Int. Health* 2001, 6, 463–475.
- [46] Ito, A., Yamasaki, H., Nakao, M., Sako, Y., Nakaya, K., Mamuti, W., Xiao, N., Sato, M. O., Ishikawa, Y., *Southeast Asian J. Trop. Med. Public Health* 2003, 34 Suppl 2, 103–107.
- [47] Georges, S., Villard, O., Filisetti, D., Mathis, A., Marcellin, L., Hansmann, Y., Candolfi, E., *J. Clin. Microbiol.* 2004, 42, 5954–5956.
- [48] Mathis, A., Deplazes, P., *Parasitol. Int.* 2006, 55 Suppl, S87–90.
- [49] Yang, Y. R., Sun, T., Zhang, J. Z., McManus, D. P., *J. Parasitol.* 2006, 92, 206–208.
- [50] Yamasaki, H., Nakaya, K., Nakao, M., Sako, Y. A. I., *Trop. Med. Health* 2007, 35, 307–321.
- [51] Elder, J. F., Jr., Turner, B. J., *Q. Rev. Biol.* 1995, 70, 297–320.
- [52] Gasser, R. B., Zhu, X. Q., Chilton, N. B., Newton, L. A., Nedergaard, T., Guldberg, P., *Electrophoresis* 1998, 19, 2391–2395.
- [53] Arnheim, N., in: M., N., K., K. R. (Eds.), *Evolution of Genes and Proteins* Sinauer, Sunderland, Massachusetts 1983, pp. 38–61.
- [54] Dover, G. A., *Genetics* 1989, 122, 249–252.
- [55] Hancock, J. M., Tautz, D., Dover, G. A., *Mol. Biol. Evol.* 1989, 5, 393–414.
- [56] Hancock, J. M., Dover, G. A., *Nucleic Acids Res.* 1990, 18, 5949–5954.
- [57] Schlotterer, C., Tautz, D., *Current Biol.* 1994, 4, 777–783.
- [58] Chilton, N. B., *Anim. Health Res. Rev.* 2001, 5, 173–187.
- [59] Chilton, N. B., Huby-Chilton, F., Gasser, R. B., *Mol. Cell. Probes* 2003, 17, 33–39.
- [60] Padgett, K. A., Nadler, S. A., Munson, L., Sacks, B., Boyce, W. M., *J. Parasitol.* 2005, 91, 1435–1443.
- [61] Hancock, K., Broughel, D. E., Moura, I. N., Khan, A., Pieniazek, N. J., Gonzalez, A. E., Garcia, H. H., Gilman, R. H., Tsang, V. C., *Int. J. Parasitol.* 2001, 31, 1601–1607.
- [62] Rinder, H., Rausch, R. L., Takahashi, K., Kopp, H., Thomschke, A., Loscher, T., *J. Parasitol.* 1997, 83, 1045–1050.
- [63] Rosenzvit, M. C., Zhang, L. H., Kamenetzky, L., Canova, S. G., Guarnera, E. A., McManus, D. P., *Parasitology* 1999, 118 (Pt 5), 523–530.
- [64] van Herwerden, L., Gasser, R. B., Blair, D., *Int. J. Parasitol.* 2000, 30, 157–169.
- [65] Utuk, A. E., Simsek, S., Koroglu, E., McManus, D. P., *Acta Trop.* 2008, 107, 192–194.
- [66] Welsh, J., McClelland, M., *Nucleic Acids Res.* 1990, 18, 7213–7218.
- [67] Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., Tingey, S. V., *Nucleic Acids Res.* 1990, 18, 6531–6535.
- [68] Siles-Lucas, M., Cuesta-Bandera, C., *J. Helminthol.* 1996, 70, 253–257.
- [69] Siles-Lucas, M., Felleisen, R., Cuesta-Bandera, C., Gottstein, B., Eckert, J., *Appl. Parasitol.* 1994, 35, 107–117.
- [70] Ortona, E., Margutti, P., Rigano, R., Siracusano, A., *Appl. Parasitol.* 1996, 37, 205–208.
- [71] Reddy, Y. A., Rao, J. R., Butchaiah, G., Sharma, B., *Vet. Parasitol.* 1998, 79, 315–323.
- [72] Azab, M. E., Bishara, S. A., Helmy, H., Oteifa, N. M., El-Hoseiny, L. M., Ramzy, R. M., Ahmed, M. A., *J. Egypt. Soc. Parasitol.* 2004, 34, 83–96.
- [73] Maravilla, P., Andrew Thompson, R. C., Palacios-Ruiz, J. A., Estcourt, A., Ramirez-Solis, E., Mondragon-de-la-Pena, C., Moreno-Moller, M., Cardenas-Mejia, A., Mata-Miranda, P., Aguirre-Alcantara, M. T., Bonilla-Rodriguez, C., Flisser, A., *Acta Trop.* 2004, 92, 231–236.
- [74] Ellsworth, D. L., Rittenhouse, K. D., Honeycutt, R. L., *BioTechniques* 1993, 14, 214–217.
- [75] MacPherson, J. M., Eckstein, P. E., Scoles, G. J., Gajadar, A. A., *Mol. Cell. Probes* 1993, 7, 293–299.
- [76] Dias, A. K., Aoki, S. M., Garcia, J. F., Nunes, C. M., *Exp. Parasitol.* 2007, 117, 9–12.
- [77] Tautz, D., *Nucleic Acids Res.* 1989, 17, 6463–6471.
- [78] Tautz, D., *EXS* 1993, 67, 21–28.
- [79] Bartholomei-Santos, M. L., Heinzelmann, L. S., Oliveira, R. P., Chemale, G., Gutierrez, A. M., Kamenetzky, L., Haag, K. L., Zaha, A., *Parasitology* 2003, 126, 599–605.
- [80] Rosenzvit, M. C., Canova, S. G., Kamenetzky, L., Guarnera, E. A., *Parasitology* 2001, 123, 381–388.
- [81] Roratto, P. A., Bartholomei-Santos, M. L., Gutierrez, A. M., Kamenetzky, L., Rosenzvit, M. C., Zaha, A., *Genet. Mol. Res.* 2006, 5, 542–552.
- [82] Maillard, S., Gottstein, B., Haag, K. L., Ma, S., Colovic, I., Benchikh-Elfegoun, M. C., Knapp, J., Piarroux, R., *J. Clin. Microbiol.* 2009, 47, 3608–3616.
- [83] Nakao, M., Sako, Y., Ito, A., *Infect. Genet. Evol.* 2003, 3, 159–163.
- [84] Bretagne, S., Assouline, B., Vidaud, D., Houin, R., Vidaud, M., *Exp. Parasitol.* 1996, 82, 324–328.
- [85] Bart, J. M., Knapp, J., Gottstein, B., El-Garch, F., Giraudoux, P., Glowatzki, M. L., Berthoud, H., Maillard, S., Piarroux, R., *Infect. Genet. Evol.* 2006, 6, 390–400.
- [86] Casulli, A., Bart, J. M., Knapp, J., La Rosa, G., Dusher, G., Gottstein, B., Di Cerbo, A., Manfredi, M. T., Genchi, C., Piarroux, R., Pozio, E., *Int. J. Parasitol.* 2009, 39, 837–842.
- [87] Knapp, J., Guislain, M. H., Bart, J. M., Raoul, F., Gottstein, B., Giraudoux, P., Piarroux, R., *Infect.*

- Genet. Evol.* 2008, 8, 367–373.
- [88] Knapp, J., Bart, J. M., Giraudoux, P., Glowatzki, M. L., Breyer, I., Raoul, F., Deplazes, P., Duscher, G., Martinek, K., Dubinsky, P., Guislain, M. H., Cliquet, F., Romig, T., Malczewski, A., Gottstein, B., Piarroux, R., *PLoS Negl. Trop. Dis.* 2009, 3, e452.
- [89] Knapp, J., Bart, J. M., Glowatzki, M. L., Ito, A., Gerard, S., Maillard, S., Piarroux, R., Gottstein, B., *J. Clin. Microbiol.* 2007, 45, 2943–2950.
- [90] Knapp, J., Bart, J. M., Maillard, S., Gottstein, B., Piarroux, R., *Parasitology* 2010, 137, 439–449.
- [91] Knapp, J., Chirica, M., Simonnet, C., Grenouillet, F., Bart, J. M., Sako, Y., Itoh, S., Nakao, M., Ito, A., Millon, L., *Emerg. Infect. Dis.* 2009, 15, 2029–2031.
- [92] Knapp, J., Staebler, S., Bart, J. M., Stien, A., Yoccoz, N. G., Drogemuller, C., Gottstein, B., Deplazes, P., *Infect. Genet. Evol.* 2012, 12, 1270–1274.
- [93] Haag, K. L., Zaha, A., Araujo, A. M., Gottstein, B., *Parasitology* 1997, 115 (Pt 5), 521–529.
- [94] Jiang, W., Liu, N., Zhang, G., Renqing, P., Xie, F., Li, T., Wang, Z., Wang, X., *Parasitol. Res.* 2012, 111, 1531–1539.
- [95] Onac, D., Gyorke, A., Oltean, M., Gavrea, R., Cozma, V., *Vet. Parasitol.* 2012.
- [96] Gholami, S., Sosari, M., Fakhar, M., Sharif, M., Daryani, A., Hashemi, M., Vahadi, M., *Iran. J. Parasitol.* 2012, 7, 8–16.
- [97] Mitrea, I. L., Ionita, M., Wassermann, M., Solcan, G., Romig, T., *Foodborne Pathog. Dis.* 2012, 9, 980–985.
- [98] Addy, F., Alakonya, A., Wamae, N., Magambo, J., Mbae, C., Mulinge, E., Zeyhle, E., Wassermann, M., Kern, P., Romig, T., *Parasitol. Res.* 2012, 111, 2289–2294.
- [99] MRad, S., Filisetti, D., Oudni, M., Mekki, M., Belguith, M., Nouri, A., Sayadi, T., Lahmar, S., Candolfi, E., Azaiez, R., Mezhoud, H., Babba, H., *Vet. Parasitol.* 2005, 129, 267–272.
- [100] Jiang, W., Wang, X., Li, M., Wang, Z., *Mol. Ecol. Resour.* 2011, 11, 206–210.
- [101] Buidakov, V. M., Aslaev, A. N., Gumerov, A. A., Tuigunov, M. M., Lukmanova, G. I., *Med. Parazitol. (Mosk)* 2010, 3–5.
- [102] Shahnazi, M., Hejazi, H., Salehi, M., Andalib, A. R., *Acta Trop.* 2011, 117, 47–50.
- [103] Tawfeek, G. M., Elwakil, H. S., Awad, N. S., El-Hoseiny, L., Thabet, H. S., Sarhan, R. M., Darweesh, S. K., Anwar, W. A., *Korean J. Parasitol.* 2009, 47, 259–264.
- [104] Arikoglu, H., Arslan, A., Hepdogru, M. A., Turhan, A. B., *Vet. Parasitol.* 2009, 166, 80–85.
- [105] Han, X. M., Wang, H., Cai, H. X., Ma, X., Liu, Y. F., Wei, B. H., Ito, A., Craig, P. S., *Chinese J. Parasitol. Parasit. Dis.* 2009, 27, 22–26.
- [106] Manterola, C., Benavente, F., Melo, A., Vial, M., Roa, J. C., *Parasitol. Int.* 2008, 57, 342–346.
- [107] Li, T., Ito, A., Nakaya, K., Qiu, J., Nakao, M., Zhen, R., Xiao, N., Chen, X., Giraudoux, P., Craig, P. S., *Trans. R. Soc. Trop. Med. Hyg.* 2008, 102, 585–590.
- [108] Villalobos, N., Gonzalez, L. M., Morales, J., de Aluja, A. S., Jimenez, M. I., Blanco, M. A., Harrison, L. J., Parkhouse, R. M., Garate, T., *Vet. Parasitol.* 2007, 147, 185–189.
- [109] Xiao, N., Nakao, M., Qiu, J., Budke, C. M., Giraudoux, P., Craig, P. S., Ito, A., *Am. J. Trop. Med. Hyg.* 2006, 75, 292–294.
- [110] Moks, E., Jogisalu, I., Saarma, U., Talvik, H., Jarvis, T., Valdmann, H., *J. Wildl. Dis.* 2006, 42, 359–365.
- [111] Zanini, F., Gonzalo, R., Perez, H., Aparici, I., Soto, X., Guerrero, J., Cerrone, G., Elissondo, C., *Vet. Parasitol.* 2006, 138, 377–381.
- [112] Ahmadi, N., Dalimi, A., *Infect. Genet. Evol.* 2006, 6, 85–90.
- [113] Nikulina, N. A., Benediktov, I., Garaev, M. M., *Med. Parazitol. (Mosk)* 2003, 29–32.
- [114] Gonzalez, L. M., Daniel-Mwambete, K., Montero, E., Rosenzvit, M. C., McManus, D. P., Garate, T., Cuesta-Bandera, C., *Exp. Parasitol.* 2002, 102, 46–56.
- [115] Harandi, M. F., Hobbs, R. P., Adams, P. J., Mobedi, I., Morgan-Ryan, U. M., Thompson, R. C., *Parasitology* 2002, 125, 367–373.
- [116] Snabel, V., D'Amelio, S., Mathiopoulos, K., Turcekova, L., Dubinsky, P., *J. Helminthol.* 2000, 74, 177–181.
- [117] Bowles, J., Blair, D., McManus, D. P., *Parasitology* 1995, 110 (Pt 3), 317–328.
- [118] McManus, D. P., Ding, Z., Bowles, J., *Acta Trop.* 1994, 56, 7–14.
- [119] Haag, K. L., Araujo, A. M., Gottstein, B., Siles-Lucas, M., Thompson, R. C., Zaha, A., *Parasitology* 1999, 118 (Pt 1), 63–71.
- [120] Haag, K. L., Araujo, A. M., Gottstein, B., Zaha, A., *Mem. Inst. Oswaldo Cruz* 1998, 93, 695–702.
- [121] Nakao, M., Yanagida, T., Okamoto, M., Knapp, J., Nkouawa, A., Sako, Y., Ito, A., *Infect. Genet. Evol.* 2010, 10, 444–452.
- [122] Jabbar, A., Narankhajid, M., Nolan, M. J., Jex, A. R., Campbell, B. E., Gasser, R. B., *Mol. Cell. Probes* 2011, 25, 49–54.
- [123] Gasser, R. B., *Int. J. Parasitol.* 1997, 27, 1449–1463.
- [124] Conole, J. C., Chilton, N. B., Jarvis, T., Gasser, R. B., *Int. J. Parasitol.* 1999, 29, 1935–1940.

- [125] Orita, M., Suzuki, Y., Sekiya, T., Hayashi, K., *Genomics* 1989, 5, 874–879.
- [126] Gasser, R. B., Hu, M., Chilton, N. B., Campbell, B. E., Jex, A. J., Otranto, D., Cafarchia, C., Beveridge, I., Zhu, X., *Nat. Protoc.* 2006, 1, 3121–3128.
- [127] Zhu, X. Q., Gasser, R. B., *Electrophoresis* 1998, 19, 1366–1373.
- [128] Gasser, R. B., Zhu, X., McManus, D. P., *Int. J. Parasitol.* 1998, 28, 1775–1779.
- [129] Sharbatkhori, M., Mirhendi, H., Jex, A. R., Pangasa, A., Campbell, B. E., Kia, E. B., Eshraghian, M. R., Harandi, M. F., Gasser, R. B., *Electrophoresis* 2009, 30, 2648–2655.
- [130] Abushhewa, M. H., Abushhiwa, M. H., Nolan, M. J., Jex, A. R., Campbell, B. E., Jabbar, A., Gasser, R. B., *Mol. Cell. Probes* 2010, 24, 346–351.
- [131] Zhang, L., Gasser, R. B., Zhu, X., McManus, D. P., *Trans. R. Soc. Trop. Med. Hyg.* 1999, 93, 329–334.
- [132] Gasser, R. B., Zhu, X., McManus, D. P., *Acta Trop.* 1998, 71, 107–115.
- [133] Oudni-Mrad, M., Cabaret, J., MRad, S., Bouzid, W., Mekki, M., Belguith, M., Sayadi, T., Nouri, A., Lahmar, S., Azaiez, R., Mezhoud, H., Babba, H., *Parasite* 2006, 13, 131–136.
- [134] Kamenetzky, L., Gutierrez, A. M., Canova, S. G., Haag, K. L., Guarnera, E. A., Parra, A., Garcia, G. E., Rosenzvit, M. C., *Infect. Genet. Evol.* 2002, 2, 129–136.
- [135] Badaraco, J. L., Ayala, F. J., Bart, J. M., Gottstein, B., Haag, K. L., *Exp. Parasitol.* 2008, 119, 453–459.
- [136] Simsek, S., Kaplan, M., Ozercan, I. H., *Parasitol. Res.* 2011, 109, 411–416.
- [137] Chemale, G., Haag, K. L., Ferreira, H. B., Zaha, A., *Mol. Biochem. Parasitol.* 2001, 116, 233–237.
- [138] Kamenetzky, L., Muzulin, P. M., Gutierrez, A. M., Angel, S. O., Zaha, A., Guarnera, E. A., Rosenzvit, M. C., *Parasitology* 2005, 131, 805–815.
- [139] Muzulin, P. M., Kamenetzky, L., Gutierrez, A. M., Guarnera, E. A., Rosenzvit, M. C., *Exp. Parasitol.* 2008, 118, 156–164.
- [140] Simsek, S., Ozcetin, C., Balkaya, I., *Vet. Parasitol.* 2012, 184, 352–355.
- [141] Le, T. H., Pearson, M. S., Blair, D., Dai, N., Zhang, L. H., McManus, D. P., *Parasitology* 2002, 124, 97–112.
- [142] Lavikainen, A., Lehtinen, M. J., Laaksonen, S., Agren, E., Oksanen, A., Meri, S., *Parasitology* 2006, 133, 565–570.
- [143] Eckert, J., Deplazes, P., Craig, P. S., Gemmell, M. A., Gottstein, B., Heath, D., Jenkins, D. J., Kamiya, M., Lightowlers, M., in: Eckert, J., Gemmell, M. A., Meslin, F. X., Pawłowski, Z. S. (Eds.), *Manual on Echinococcus in Humans and Animals: A Public Health Problem of Global Concern*, WHO/OIE, Paris, France 2001, pp. 73–100.
- [144] Craig, P. S., McManus, D. P., Lightowlers, M. W., Chabalgoity, J. A., Garcia, H. H., Gavidia, C. M., Gilman, R. H., Gonzalez, A. E., Lorca, M., Naquira, C., Nieto, A., Schantz, P. M., *Lancet Infect. Dis.* 2007, 7, 385–394.
- [145] Nakao, M., Yanagida, T., Okamoto, M., Knapp, J., Nkouawa, A., Sako, Y., Ito, A., *Infect. Genet. Evol.* 2010, 10, 444–452.
- [146] Kedra, A. H., Swiderski, Z., Tkach, V. V., Dubinsky, P., Pawłowski, Z., Stefaniak, J., Pawłowski, J., *Acta Parasitol.* 1999, 44, 248–254.
- [147] Saarma, U., Jogisalu, I., Moks, E., Varcasia, A., Lavikainen, A., Oksanen, A., Simsek, S., Andresiuk, V., Denegri, G., Gonzalez, L. M., Ferrer, E., Garate, T., Rinaldi, L., Maravilla, P., *Parasitology* 2009, 136, 317–328.
- [148] Huttner, M., Nakao, M., Wassermann, T., Siefert, L., Boomker, J. D., Dinkel, A., Sako, Y., Mackenstedt, U., Romig, T., Ito, A., *Int. J. Parasitol.* 2008, 38, 861–868.
- [149] Righetti, P. G., Gelfi, C., D'Acunto, M. R., *Electrophoresis* 2002, 23, 1361–1374.
- [150] Bjorheim, J., Ekstrom, P. O., *Electrophoresis* 2005, 26, 2520–2530.
- [151] Mitchelson, K. R., *Mol. Biotechnol.* 2003, 24, 41–68.
- [152] Doi, K., Doi, H., Noiri, E., Nakao, A., Fujita, T., Tokunaga, K., *Electrophoresis* 2004, 25, 833–838.
- [153] Jespersgaard, C., Larsen, L. A., Baba, S., Kukita, Y., Tahira, T., Christiansen, M., Vuust, J., Hayashi, K., Andersen, P. S., *Electrophoresis* 2006, 27, 3816–3822.
- [154] Chung, J. H., Park, Y. S., Kim, J., Shin, G. W., Nam, M. H., Oh, M. K., Kim, C. W., Jung, G. Y., Hyun Park, J., *Electrophoresis* 2007, 28, 2416–2423.
- [155] Zinger, L., Gury, J., Alibeu, O., Rioux, D., Gielly, L., Sage, L., Pompanon, F., Geremia, R. A., *J. Microbiol. Methods* 2008, 72, 42–53.
- [156] Hawkins, G. A., Hoffman, L. M., *Nat. Biotechnol.* 1997, 15, 803–804.
- [157] Yamasaki, H., Nakao, M., Sako, Y., Nakaya, K., Sato, M. O., Mamuti, W., Okamoto, M., Ito, A., *J. Clin. Microbiol.* 2002, 40, 3818–3821.
- [158] Wittwer, C. T., *Hum. Mutat.* 2009, 30, 857–859.
- [159] Rasmussen, J. P., Saint, C. P., Monis, P. T., *BMC Bioinformatics* 2007, 8, 107–117.
- [160] Robinson, B. S., Monis, P. T., Dobson, P. J., *Appl. Environ. Microbiol.* 2006, 72, 5857–5863.
- [161] Kirkpatrick, N. C., Blacker, H. P., Woods, W. G., Gasser, R. B., Noormohammadi, A. H., *Avian Pathol.* 2009, 38, 13–19.
- [162] Pangasa, A., Jex, A. R., Campbell, B. E., Bott, N. J., Whipp, M., Hogg, G., Stevens, M. A., Gasser, R.

- B., *Mol. Cell. Probes* 2009, 23, 10–15.
- [163] Gasser, R. B., *Vet. Parasitol.* 2013, *In Press*.
- [164] Vajda, E., Tarjan, E., Gonda, G., Zsiray, M., Appel, J., Farkas, E., Somoskovi, A., *Orv. Hetil.* 1995, 136, 1787–1790.
- [165] Bentley, D. R., Balasubramanian, S., Swerdlow, H. P., Smith, G. P., Milton, J., Brown, C. G., Hall, K. P., Evers, D. J., Barnes, C. L., Bignell, H. R., Boutell, J. M., Bryant, J., Carter, R. J., Keira Cheetham, R., Cox, A. J., Ellis, D. J., Flatbush, M. R., Gormley, N. A., Humphray, S. J., Irving, L. J., Karbelaishvili, M. S., Kirk, S. M., Li, H., Liu, X., Maisinger, K. S., Murray, L. J., Obradovic, B., Ost, T., Parkinson, M. L., Pratt, M. R., Rasolonjatovo, I. M., Reed, M. T., Rigatti, R., Rodighiero, C., Ross, M. T., Sabot, A., Sankar, S. V., Scally, A., Schroth, G. P., Smith, M. E., Smith, V. P., Spiridou, A., Torrance, P. E., Tzonev, S. S., Vermaas, E. H., Walter, K., Wu, X., Zhang, L., Alam, M. D., Anastasi, C., Aniebo, I. C., Bailey, D. M., Bancarz, I. R., Banerjee, S., Barbour, S. G., Baybayan, P. A., Benoit, V. A., Benson, K. F., Bevis, C., Black, P. J., Boodhun, A., Brennan, J. S., Bridgham, J. A., Brown, R. C., Brown, A. A., Buermann, D. H., Bundu, A. A., Burrows, J. C., Carter, N. P., Castillo, N., Chiara, E. C. M., Chang, S., Neil Cooley, R., Crake, N. R., Dada, O. O., Diakoumakos, K. D., Dominguez-Fernandez, B., Earnshaw, D. J., Egbujor, U. C., Elmore, D. W., Etchin, S. S., Ewan, M. R., Fedurco, M., Fraser, L. J., Fuentes Fajardo, K. V., Scott Furey, W., George, D., Gietzen, K. J., Goddard, C. P., Golda, G. S., Granieri, P. A., Green, D. E., Gustafson, D. L., Hansen, N. F., Harnish, K., Haudenschild, C. D., Heyer, N. I., Hims, M. M., Ho, J. T., Horgan, A. M., Hoschler, K., Hurwitz, S., Ivanov, D. V., Johnson, M. Q., James, T., Huw Jones, T. A., Kang, G. D., Kerelska, T. H., Kersey, A. D., Khrebtukova, I., Kindwall, A. P., Kingsbury, Z., Kokko-Gonzales, P. I., Kumar, A., Laurent, M. A., Lawley, C. T., Lee, S. E., Lee, X., Liao, A. K., Loch, J. A., Lok, M., Luo, S., Mammen, R. M., Martin, J. W., McCauley, P. G., McNitt, P., Mehta, P., Moon, K. W., Mullens, J. W., Newington, T., Ning, Z., Ling Ng, B., Novo, S. M., O'Neill, M. J., Osborne, M. A., Osnowski, A., Ostadan, O., Paraschos, L. L., Pickering, L., Pike, A. C., Pike, A. C., Chris Pinkard, D., Pliskin, D. P., Podhasky, J., Quijano, V. J., Racz, C., Rae, V. H., Rawlings, S. R., Chiva Rodriguez, A., Roe, P. M., Rogers, J., Rogert Bacigalupo, M. C., Romanov, N., Romieu, A., Roth, R. K., Rourke, N. J., Ruediger, S. T., Rusman, E., Sanches-Kuiper, R. M., Schenker, M. R., Seoane, J. M., Shaw, R. J., Shiver, M. K., Short, S. W., Sizto, N. L., Sluis, J. P., Smith, M. A., Ernest Sohna Sohna, J., Spence, E. J., Stevens, K., Sutton, N., Szajkowski, L., Tregidgo, C. L., Turcatti, G., Vandevondele, S., Verhovsky, Y., Virk, S. M., Wakelin, S., Walcott, G. C., Wang, J., Worsley, G. J., Yan, J., Yau, L., Zuerlein, M., Rogers, J., Mullikin, J. C., Hurles, M. E., McCooke, N. J., West, J. S., Oaks, F. L., Lundberg, P. L., Klenerman, D., Durbin, R., Smith, A. J., *Nature* 2008, 456, 53–59.
- [166] *Schistosoma japonicum* Genome, S., Functional Analysis, C., *Nature* 2009, 460, 345–351.
- [167] Berriman, M., Haas, B. J., LoVerde, P. T., Wilson, R. A., Dillon, G. P., Cerqueira, G. C., Mashiyama, S. T., Al-Lazikani, B., Andrade, L. F., Ashton, P. D., Aslett, M. A., Bartholomeu, D. C., Blandin, G., Caffrey, C. R., Coghlan, A., Coulson, R., Day, T. A., Delcher, A., DeMarco, R., Djikeng, A., Eyre, T., Gamble, J. A., Ghedin, E., Gu, Y., Hertz-Fowler, C., Hirai, H., Hirai, Y., Houston, R., Ivens, A., Johnston, D. A., Lacerda, D., Macedo, C. D., McVeigh, P., Ning, Z., Oliveira, G., Overington, J. P., Parkhill, J., Pertea, M., Pierce, R. J., Protasio, A. V., Quail, M. A., Rajandream, M. A., Rogers, J., Sajid, M., Salzberg, S. L., Stanke, M., Tivey, A. R., White, O., Williams, D. L., Wortman, J., Wu, W., Zamanian, M., Zerlotini, A., Fraser-Liggett, C. M., Barrell, B. G., El-Sayed, N. M., *Nature* 2009, 460, 352–358.
- [168] Young, N. D., Jex, A. R., Li, B., Liu, S., Yang, L., Xiong, Z., Li, Y., Cantacessi, C., Hall, R. S., Xu, X., Chen, F., Wu, X., Zerlotini, A., Oliveira, G., Hofmann, A., Zhang, G., Fang, X., Kang, Y., Campbell, B. E., Loukas, A., Ranganathan, S., Rollinson, D., Rinaldi, G., Brindley, P. J., Yang, H., Wang, J., Wang, J., Gasser, R. B., *Nat. Genet.* 2012, 44, 221–225.
- [169] Mardis, E. R., *Ann. Rev. Genom. Human Genet.* 2008, 9, 387–402.
- [170] Morozova, O., Marra, M. A., *Genomics* 2008, 92, 255–264.
- [171] Cantacessi, C., Campbell, B. E., Gasser, R. B., *Biotechnol. Adv.* 2012, 30, 469–488.
- [172] Cantacessi, C., Campbell, B. E., Jex, A. R., Hall, R. S., Young, N. D., Nolan, M. J., Gasser, R. B., in: Caffrey, C., Selzer, P. M. (Eds.), *Parasitic Helminths: Targets, Screens, Drugs and Vaccines. Drug Discovery in Infectious Diseases*, WILEY-VCH Verlag & Co. KGaA, Weinheim, Germany 2012, pp. 61–72.
- [173] Young, N. D., Campbell, B. E., Hall, R. S., Jex, A. R., Cantacessi, C., Laha, T., Sohn, W. M., Sripa, B., Loukas, A., Brindley, P. J., Gasser, R. B., *PLoS Negl. Trop. Dis.* 2010, 4, e719.
- [174] Young, N. D., Hall, R. S., Jex, A. R., Cantacessi, C., Gasser, R. B., *Biotechnol. Adv.* 2010, 28, 222–231.
- [175] Jex, A. R., Liu, S., Li, B., Young, N. D., Hall, R. S., Li, Y., Yang, L., Zeng, N., Xu, X., Xiong, Z., Chen, F., Wu, X., Zhang, G., Fang, X., Kang, Y., Anderson, G. A., Harris, T. W., Campbell, B. E., Vlaminc, J., Wang, T., Cantacessi, C., Schwarz, E. M., Ranganathan, S., Geldhof, P., Nejsun, P., Sternberg, P. W., Yang, H., Wang, J., Wang, J., Gasser, R. B., *Nature* 2011, 479, 529–533.
- [176] Metzker, M. L., *Nat. Rev. Genet.* 2010, 11, 31–46.
- [177] 1000 Genomes Project Consortium, Abecasis, G. R., Auton, A., Brooks, L. D., DePristo, M. A.,

- Durbin, R. M., Handsaker, R. E., Kang, H. M., Marth, G. T., McVean, G. A., *Nature* 2012 491, 56-65.
- [178] Olson, P. D., Zarowiecki, M., Kiss, F., Brehm, K., *Parasite Immunol.* 2012, 34, 130–150.
- [179] Jex, A. R., Hu, M., Littlewood, D. T., Waeschenbach, A., Gasser, R. B., *BMC Genomics* 2008, 9, 11.
- [180] Jex, A. R., Hall, R. S., Littlewood, D. T., Gasser, R. B., *Nucleic Acids Res.* 2010, 38, 522–533.
- [181] ten Bosch, J. R., Grody, W. W., *J. Mol. Diagn.* 2008, 10, 484–492.
- [182] Gupta, P. K., *Trends Biotechnol.* 2008, 26, 602–611.
- [183] Yates, L. R., Campbell, P. J., *Nat. Rev. Genet.* 2012, 13, 795–806.
- [184] Weinstock, G. M., *Nature* 2012, 489, 250–256.
- [185] Parra, G., Bradnam, K., Korf, I., *Bioinformatics* 2007, 23, 1061–1067.

Table 1. Currently recognized species of *Echinococcus*

Species	Main definitive hosts	Main intermediate hosts ^a	Larval form	Principal location of larva	Geographical distribution ^c
<i>Echinococcus granulosus</i>	Canids	Sheep, goat, cattle, other herbivores, pig, human	Unilocular cyst	Liver, lungs	Cosmopolitan
<i>E. multilocularis</i>	Canids, felids	Rodents; human	Multilocular cyst ^b	Liver	Central and northern Eurasia, North America
<i>E. oligarthrus</i>	Felids	Rodents; human	Polycystic	Muscles	Central and South America
<i>E. shiquicus</i>	Tibetan fox (<i>Vulpes ferrilata</i>)	Lagomorph (<i>Ochotona curzoniae</i>)	Unilocular cyst	Liver, lungs	Tibetan Plateau
<i>E. vogeli</i>	Canids	Rodents; human	Polycystic	Liver	Central and South America

^a Humans are accidental hosts.

^b Larval stage is metastatic.

^c Key references: [8-11]

Table 2. Currently recognized genotypes of *Echinococcus granulosus*

Genotype / Main host (proposed species designation ^a)	Main definitive hosts	Main intermediate Hosts^b	Original report
G1/Sheep	Canids	Sheep, other ruminants, macropods, humans	[21]
G2 / Tasmanian sheep	Canids	Sheep, humans	[21]
G3 / Buffalo	Canids	Buffalo, humans	[21]
G4 / Horse (<i>E. equinus</i>)	Canids	Horse	[21]
G5 / Cattle (<i>E. ortleppi</i>)	Canids	Cattle, humans	[21]
G6 / Camel (<i>E. canadensis</i>)	Canids	Camel, humans	[21]
G7 / Pig (<i>E. canadensis</i>)			[21]
G8 / Cervid (<i>E. canadensis</i>)	Canids	Cervids	[22]
G9 ^c / Human	Canids (?)	Human (?)	[29]
G10 / Cervid (<i>E. canadensis</i>)	Canids	Cervids	[30]

^a Ref. [10]^b Humans are accidental hosts.^c Status is controversial.

Table 3. Main applications of single-strand conformation polymorphism (SSCP)-based methods to explore genetic variation within and among different species of *Echinococcus*

Application	Species	Genetic loci in mitochondrial or nuclear DNA ^a	References
Population genetic or epidemiology studies	<i>Echinococcus granulosus</i>	<i>cox1</i>	[128]
	<i>E. granulosus</i>	<i>cox1, nad1</i> <i>cox1, Ag4, Ag6</i>	[122, 129-132] [133]
	<i>E. granulosus</i>	<i>cox1, mdh</i> <i>AgB4-1, AgB4-2, cox1, mdh1-3</i>	[134] [135]
	<i>E. multilocularis</i>	<i>cox1, 12S rRNA</i> <i>AgB, nad1, ActII, Hbx2</i>	[136] [93]
Assessing genetic variation within the gene family encoding Ag B	<i>E. granulosus</i>	<i>AgB</i>	[137]
	<i>E. granulosus</i>	<i>AgB</i> <i>AgB gene family</i> <i>AgB1</i>	[138] [139] [140]
Study of the breeding system	<i>E. granulosus</i> , <i>E. multilocularis</i> and <i>E. vogeli</i>	<i>AgB, ActII, Hbx2, nad1</i>	[120]
	<i>E. granulosus</i>	<i>ActII, AgB1, Ag4, Ag6, Hbx2, nad1</i>	[119]

^a Representing *ActII*, actinII; *AgB*, Antigen B complex; *AgB1*, variant one of Antigen B; *AgB2*, variant two of Antigen B; *Ag4*, antigen 4 - cytosolic malate dehydrogenase; *Ag6*, antigen 6 - calcium-binding protein; *cox1*, mitochondrial cytochrome *c* oxidase subunit I; *Hbx2*, homeobox2; *mdh*, nuclear malate dehydrogenase; *mdh1-3*, malate dehydrogenase one, two and three; *nad1*, mitochondrial nicotinamide dehydrogenase subunit 1.