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OPEN
doi:10.1038/nature12031

# The genomes of four tapeworm species reveal adaptations to parasitism

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**Tapeworms (Cestoda) cause neglected diseases that can be fatal and are difficult to treat, owing to inefficient drugs. Here we present an analysis of tapeworm genome sequences using the human-infective species *Echinococcus multilocularis*, *E. granulosus*, *Taenia solium* and the laboratory model *Hymenolepis microstoma* as examples. The 115- to 141-megabase genomes offer insights into the evolution of parasitism. Synteny is maintained with distantly related blood flukes but we find extreme losses of genes and pathways that are ubiquitous in other animals, including 34 homeobox families and several determinants of stem cell fate. Tapeworms have specialized detoxification pathways, metabolism that is finely tuned to rely on nutrients scavenged from their hosts, and species-specific expansions of non-canonical heat shock proteins and families of known antigens. We identify new potential drug targets, including some on which existing pharmaceuticals may act. The genomes provide a rich resource to underpin the development of urgently needed treatments and control.**

Echinococcosis (hydatid disease) and cysticercosis, caused by the proliferation of larval tapeworms in vital organs[1], are among the most severe parasitic diseases in humans and account for 2 of the 17 neglected tropical diseases prioritized by the World Health Organization[2]. Larval tapeworms can persist asymptomatically in a human host for decades[3], eventually causing a spectrum of debilitating pathologies and death[1]. When diagnosed, the disease is often at an advanced stage at which surgery is no longer an option[4]. Tapeworm infections are highly prevalent worldwide[5], and their human disease burden has been estimated at 1 million disability-adjusted life years, comparable with African trypanosomiasis, river blindness and dengue fever. Furthermore, cystic echinococcosis in livestock causes an annual loss of US$2 billion[6].

Tapeworms (Platyhelminthes, Cestoda) are passively transmitted between hosts and parasitize virtually every vertebrate species[7]. Their morphological adaptations to parasitism include the absence of a gut, a head and light-sensing organs, and they possess a unique surface (tegument) that is able to withstand host-stomach acid and bile but is still penetrable enough to absorb nutrients[7].

Tapeworms are the only one of three major groups of worms that parasitize humans, the others being flukes (Trematoda) and round worms (Nematoda), for which no genome sequence has been available so far. Here we present a high-quality reference tapeworm genome of a human-infective fox tapeworm *Echinococcus multilocularis.* We also present the genomes of three other species, for comparison; *E. granulosus* (dog tapeworm), *Taenia solium* (pork tapeworm), both of which infect humans, and *Hymenolepis microstoma* (a rodent tapeworm and laboratory model for the human parasite *Hymenolepis nana*). We have mined the genomes to provide a starting point for developing urgently needed therapeutic measures against tapeworms and other parasitic flatworms. Access to the complete genomes of several tapeworms will accelerate the pace at which new tools and treatments to combat tapeworm infections can be discovered.

## The genomes and genes of tapeworms

The *E. multilocularis* genome assembly was finished manually (Supplementary Information, section 2), producing a high-quality reference

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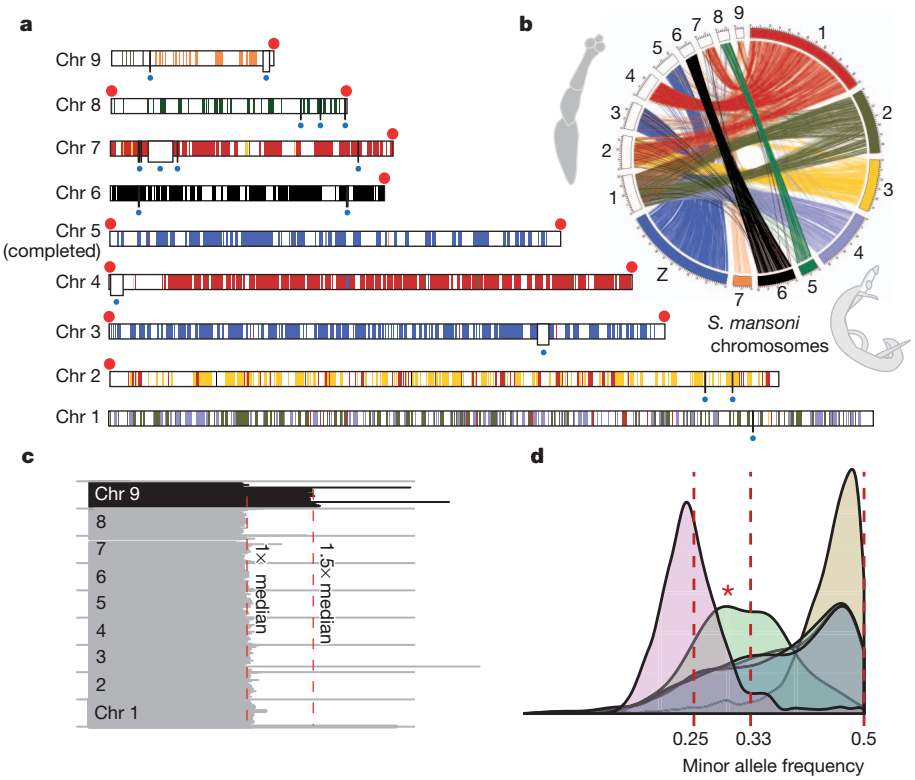



**Figure 1 | Genome of *E. multilocularis*.** **a**, The nine assembled chromosomes (Chr 1–Chr 9) of *E. multilocularis* with telomeres (red dots). Physical gaps in the sequence assembly (white boxes with blue dot beneath) are bridged by optical map data. Each colour segment is defined as an array of at least three genes that each has a single orthologous counterpart on one *S. mansoni* chromosome, regardless of their locations on the chromosome. **b**, One-to-one orthologues connecting *E. multilocularis* and *S. mansoni* chromosomes. **c**, Distribution of normalized genome coverage on isolate GT10/2. Each horizontal line depicts median coverage of 100-kb windows normalized against the mean coverage for the genome (130 × ). Even coverage was observed across the first eight chromosomes, but 1.5 × coverage of chromosome 9 indicates trisomy. Similar plots for other isolates are shown in Supplementary Fig. 3.1. **d**, Distribution of minor allele frequency (MAF) of heterozygous sites in five isolates of *E. multilocularis* (plot for individual isolates in Supplementary Fig. 3.1), identified by mapping sequencing reads against the assembled chromosome consensus sequences. At each site, the proportion of bases that disagree with the reference is counted. For four isolates, the MAF peaks at around 0.5, indicative of diploidy, whereas JAVA05/1 peaks at 0.25, suggesting tetraploidy. Chromosome 9 of GT10/2 is plotted separately (marked by asterisk) from chromosomes 1 to 8, and the MAF display a clear departure of 0.5 and peaks around 0.33, consistent with a trisomy.

genome in which 89% of the sequence is contained in 9 chromosome scaffolds that have only 23 gaps (Supplementary Table 1.2). One chromosome is complete from telomere to telomere, and 13 of the expected 18 telomeres are joined to scaffolds (Fig. 1a). This quality and completeness is comparable to that of the first publications of *Caenorhabditis elegans* and *Drosophila melanogaster* genomes[8,9]. The 115- to 141-megabase (Mb) nuclear tapeworm genomes were sequenced using several high-throughput technologies (Supplementary Table 1.1). The tapeworm genomes are approximately one-third of the size of the genome of their distant flatworm relative, the blood fluke *Schistosoma mansoni*[10], mainly because it has fewer repeats (Supplementary Information, section 3). By sequencing several isolates of *E. multilocularis* (Supplementary Table 3.2), we revealed tetraploidy in protoscoleces of one isolate, and a trisomy of chromosome 9 (the smallest chromosome, and possibly the only one for which a trisomy is tolerated) transiently exhibited in protoscoleces and metacestodes from two different isolates (Fig. 1c, d and Supplementary Figs 3.1, 3.2 and 3.3), consistent with previous observations of karyotype plasticity in flatworms[11].

Aided by deep transcriptome sequencing from multiple life-cycle stages, we identified 10,231 to 12,490 putative genes per genome (Supplementary Table 5.5). Similar to the genome of *S. mansoni*[12], distinct 'micro-exon genes' are present in tapeworm genomes, with multiple internal exons that are small (typically less than 36 bases) and divisible by 3 (Supplementary Information, section 5). To identify gene gain and loss in tapeworms, orthologous relationships were predicted between tapeworms and eight other species (Fig. 2). Although gene order has been lost, ancient chromosomal synteny is preserved among parasitic flatworms (Fig. 1b and Supplementary Table 7.3). Two chromosomes in *E. multilocularis* (Fig. 1a, b) correspond to the *S. mansoni* Z sex chromosome. Schistosomes are unusual among flatworms in that they have sexual dimorphism, but how common ancestors of both tapeworms and flukes evolved into female-heterogametic parasites, like *S. mansoni*, remains to be elucidated.

Genome-wide identification of polycistrons in tapeworms shows that there are 308 putative polycistrons in *E. multilocularis*, with the largest containing 4 genes. The internal gene order within *E. multilocularis* polycistrons is largely the same as in *T. solium* and *H. microstoma* (Supplementary Table 6.5), and—to some extent—as in flukes; 39% of *S. mansoni* orthologues of genes within *E. multilocularis* polycistrons retain colinearity. Of these *S. mansoni* genes, 40% have transcriptome evidence supporting their polycistronic transcription[10], demonstrating further that gene order in polycistrons is highly conserved over long evolutionary time[13] ($P < 0.0001$, Supplementary Information, section 6).

Polycistrons are resolved into individual coding transcripts using spliced-leader *trans*-splicing, but spliced-leader *trans*-splicing also occurs in genes outside of polycistrons. Using deep transcriptome sequencing (RNA-seq) we found evidence of spliced-leader *trans*-splicing in approximately 13% of *E. multilocularis* genes (Supplementary Table 6.2), less than the 70% observed in *C. elegans*[14] and 58% in a tunicate[15].

## Specialized metabolism and detoxification

The high-confidence gene sets reveal extensive reductions in overall metabolic capability and an increased ability to absorb nutrients, compared to that of other animals (Figs 2 and 3, and Supplementary Information, section 9). Their main energy source, carbohydrates, can be catabolized by aerobic respiration or by two complementary anaerobic pathways; the lactate fermentation and malate dismutation pathways. The parasiticidal effects of mitochondrial fumarate reductase inhibitors have been demonstrated *in vitro*, suggesting that the malate dismutation pathway would be an effective target for the development of novel therapeutics[16].

Tapeworms, like flukes, lack the ability to synthesize fatty acids and cholesterol *de novo*[17,18]. Instead, they scavenge essential fats from the host using fatty acid transporters and lipid elongation enzymes (Supplementary Table 9.2), as well as several tapeworm-specific gene families, such as fatty acid binding protein (FABP) and the apolipoprotein

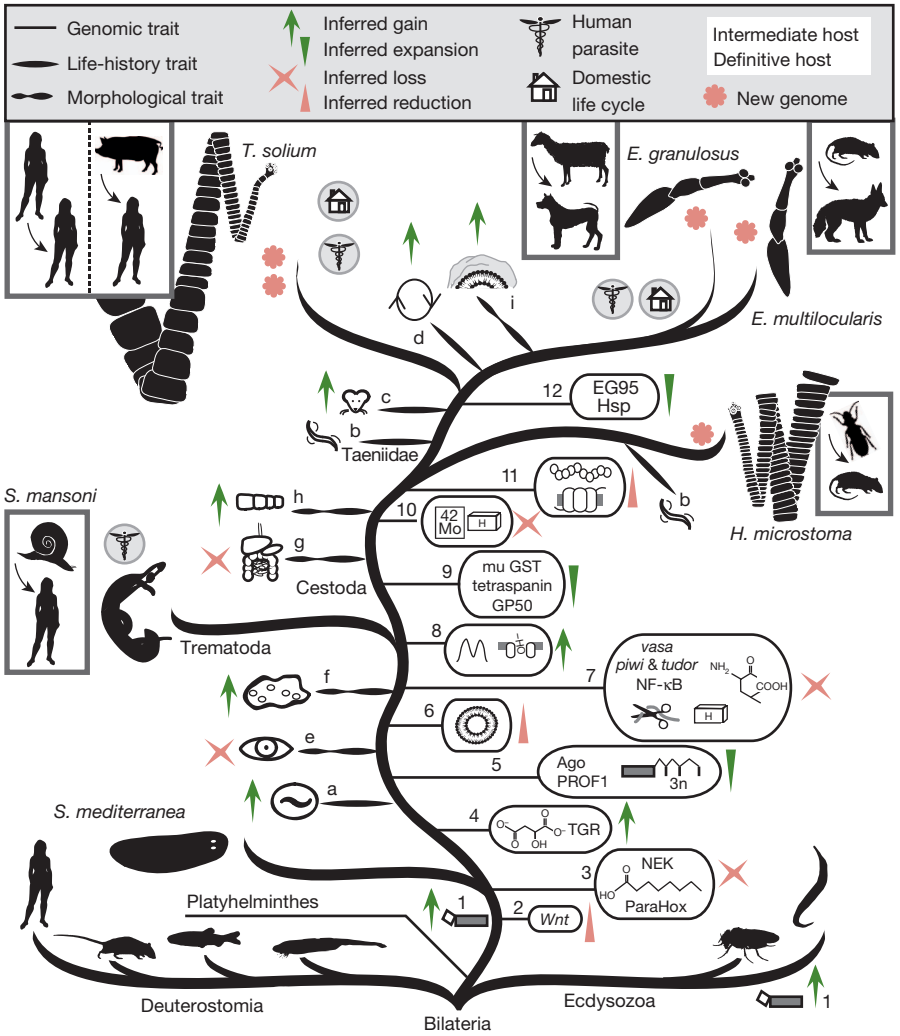



**Figure 2 | Evolution of tapeworm parasitism.** Phylogeny of the main branches of Bilateria; Ecdysozoa (including fruitflies and nematodes), Deuterostomia (including lancelet, zebrafish, mice and humans), and Lophotrochozoans (including Platyhelminthes (flatworms)) (based on phylogeny in Supplementary Fig. 7.1). The gains and losses of life-cycle traits for these parasitic flatworms include the evolution of endoparasitism (a), passive transmission between hosts (b), acquisition of vertebrate intermediate host (c), ability to proliferate asexually in intermediate host (d). Morphological traits that have evolved include the loss of eye cups (e), gain of neodermatan syncytial epithelia (f), loss of gut (g), segmentation of body plan (h), and changes in the laminated layer (to contain specialized apomucins; i). Gains and losses of genomic traits include spliced-leader *trans*-splicing (1), loss of *Wnt* genes (2), loss of NEK kinases, fatty acid biosynthesis and ParaHox genes (3), anaerobic metabolic ability through the malate dismutation/rodhoquinone pathway, merger of *glutaredoxin* and *thioredoxin reductase* to *thioredoxin glutathione reductase* (TGR) (4), evolution of tapeworm- and fluke-specific Argonaute (Ago) family, micro exon genes (MEGs) and PROF1 GPCRs (5), loss of peroxisomal genes (6), and complete loss of *vasa*, *tudor* and *piwi* genes, NF-κB pathway, loss of 24 homeobox gene families (indicated by 'H'), metabolic proteases and amino acid biosynthesis (7). In tapeworms, gains and losses of genomic traits include innovation of bimodal intron distribution and novel fatty acid transporters (8), expansion of mu-class glutathione *S*-transferases, GP50 antigens and tetraspanins (9), loss of the molybdopterin biosynthesis pathway, loss of 10 homeobox gene families (10), fewer GPCRs and fewer neuropeptides encoded by each protopeptide (11), and expansion of heat shock proteins (Hsp) and species-specific antigens (12).

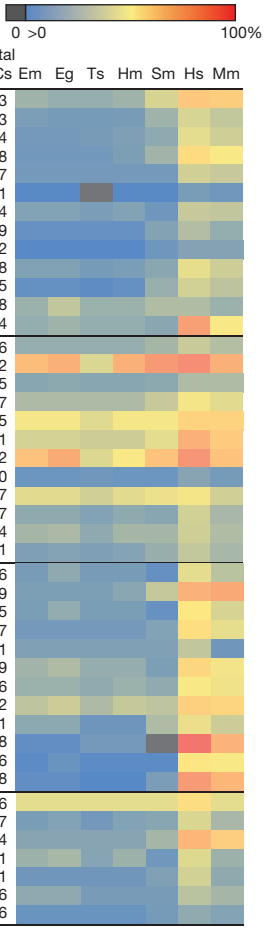
antigen B (Supplementary Information, section 8). Uptake of fatty acids seems to be crucial in *Echinococcus* spp. metacestodes, in which both FABP and antigen B gene families are among the most highly expressed genes[19] (Supplementary Table 5.7). Tapeworms and flukes have lost many genes associated with the peroxisome (Supplementary Information, section 8), an organelle in which fatty acid oxidation occurs, and may lack peroxisomes altogether, as seen in several other parasites[20].

Compared with other animals, *S. mansoni* has a reduced ability to synthesize amino acids[17]. In tapeworms, this capacity is reduced further, with serine and proline biosynthesis enzymes absent from *E. multilocularis* (Fig. 3 and Supplementary Information, section 9). Many enzymes in the molybdopterin biosynthesis pathway seemed to be lost in tapeworms, along with enzymes that use molybdopterin as a cofactor. The ability to utilize molybdenum in enzymatic reactions was believed to be present in all animals[21], but has been lost in some eukaryotic parasites[22].

Differences in the detoxification systems between tapeworms and their mammalian hosts may be exploited for drug design (Supplementary Information, section 9). We found that, like flukes[23], tapeworms typically have only one cytochrome P450 gene, suggesting that their ability to oxidize many xenobiotics and steroids is substantially lower than that of their hosts. Uniquely, tapeworms and flukes have merged two key enzymatic functions for redox homeostasis in one single enzyme: thioredoxin glutathione reductase (TGR). TGR is an essential gene and validated drug target in flukes[24]. Downstream of TGR we find an unexpected diversity of thioredoxins, glutaredoxins and mu-class glutathione S-transferases (GSTs) (Supplementary Table 9.3). The GST expansion suggests that tapeworms would be able to water-solubilize and excrete a large range of hydrophobic compounds, which may add complexity to the pharmacokinetics of drugs.

## Homeobox gene loss

Homeobox genes are high-level transcription factors that are implicated in the patterning of body plans in animals. Across parasitic flatworms, the homeobox gene numbers are extensively reduced (Supplementary Table 10.1). Most bilaterian invertebrates have a conserved set of approximately 100 homeobox genes (for example, 92 conserved in *C. elegans*, 102 in *D. melanogaster*, and 133 in the lancelet)[25]. Of the 96 homeobox gene families that are thought to have existed at the origin of the Bilateria, 24 are not present in tapeworms and flukes, and a further 10 were lost in tapeworms, making their complement by far the most reduced of any studied bilaterian animal[25]. Among the tapeworm-specific gene losses are gene families involved in neural development (*mnx*, *pax3/7*, *gbx*, *hbn* and *rax*). This is somewhat surprising considering that tapeworms possess a well-developed nervous system, albeit with reduced sensory input and cephalization. Tapeworms also lack the ParaHox genes (*gsx*, *pdx*, *cdx*) ancestrally involved in specification of a through-gut[26,27], although these seem to have been lost before the tapeworm gut was lost. Other conserved genes found in bilaterian developmental pathways such as Hedgehog and Notch were found to be present and intact, although the *Wnt* complement is greatly reduced compared to the ancestral (spiralian) complement of 12 *Wnt* ligands[28] (Supplementary Table 10.2).




| Super-pathway | Pathway | Em ECs | Total ECs |
|---|---|---|---|
| Amino acid metabolism | Alanine, aspartate and glutamate metabolism | 10 | 43 |
| | Arginine and proline metabolism | 14 | 103 |
| | Cysteine and methionine metabolism | 7 | 64 |
| | Glycine, serine and threonine metabolism | 6 | 58 |
| | Histidine metabolism | 4 | 37 |
| | Lysine biosynthesis | 1 | 31 |
| | Lysine degradation | 8 | 54 |
| | Phenylalanine metabolism | 4 | 59 |
| | Phenylalanine, tyrosine and tryptophan biosynthesis | 1 | 32 |
| | Tryptophan metabolism | 10 | 68 |
| | Tyrosine metabolism | 4 | 65 |
| | Valine, leucine and isoleucine biosynthesis | 4 | 18 |
| | Valine, leucine and isoleucine degradation | 7 | 34 |
| Carbohydrate metabolism | Amino sugar and nucleotide sugar metabolism | 20 | 96 |
| | Citrate cycle (TCA cycle) | 15 | 22 |
| | Fructose and mannose metabolism | 11 | 65 |
| | Galactose metabolism | 10 | 37 |
| | Glycolysis/gluconeogenesis | 22 | 45 |
| | Inositol phosphate metabolism | 16 | 41 |
| | Oxidative phosphorylation | 8 | 12 |
| | Pentose and glucuronate interconversions | 5 | 60 |
| | Pentose phosphatepathway | 16 | 37 |
| | Propanoate metabolism | 9 | 47 |
| | Pyruvate metabolism | 16 | 64 |
| | Starch and sucrose metabolism | 10 | 71 |
| Lipid metabolism | α-Linolenic acid metabolism | 2 | 16 |
| | Arachidonic acid metabolism | 4 | 29 |
| | Biosynthesis of unsaturated fatty acids | 2 | 15 |
| | Ether lipid metabolism | 3 | 27 |
| | Fatty acid biosynthesis | 3 | 21 |
| | Fatty acid metabolism | 7 | 29 |
| | Glycerolipid metabolism | 8 | 36 |
| | Glycerophospholipid metabolism | 17 | 52 |
| | Linoleic acid metabolism | 2 | 11 |
| | Primary bile acid biosynthesis | 1 | 18 |
| | Steroid biosynthesis | 1 | 26 |
| | Steroid hormone biosynthesis | 2 | 38 |
| Metabolism of cofactors and vitamins | Folate biosynthesis | 7 | 16 |
| | Nicotinate and nicotinamide metabolism | 6 | 47 |
| | One carbon pool by folate | 4 | 24 |
| | Pantothenate and CoA biosynthesis | 7 | 31 |
| | Riboflavin metabolism | 2 | 21 |
| | Thiamine metabolism | 3 | 16 |
| | Vitamin B6 metabolism | 2 | 26 |

**Figure 3 | Conservation of individual metabolic pathways.** Heatmap showing the conservation of individual metabolic pathways for *E. multilocularis* (Em), *E. granulosus* (Eg), *T. solium* (Ts), *H. microstoma* (Hm) and *S. mansoni* (Sm) compared to those of humans (Hs) and mice (Mm). Each row indicates an individual metabolic pathway grouped by their superclass membership (defined by KEGG (Kyoto Encyclopedia of Genes and Genomes)). Coloured tiles indicate the level of conservation (percentage of enzymes detected) of each pathway within each species. KEGG pathways with insufficient evidence (that is, containing only one enzyme) in *E. multilocularis* have been removed. CoA, coenzyme A; EC, enzyme commission number; TCA, tricarboxylic acid cycle.

## Stem cell specializations

Extreme regenerative capability and developmental plasticity, mediated by ever-present somatic stem cells (neoblasts), have made flatworms popular models for stem cell research[29]. All multicellular organisms rely on stem cells for proliferation and growth, so it is remarkable that tapeworms and flukes appear to lack the ubiquitous stem cell marker gene *vasa* (Supplementary Information, section 11). Instead tapeworms have two copies of another dead-box helicase (PL10), which we propose may have taken over some of the functions of *vasa* (Supplementary Fig. 11.1). Tapeworms and flukes are also missing the *piwi* gene subfamily and *piwi*-interacting *tudor*-domain containing proteins. The *piwi* genes belong to a subfamily of genes encoding argonaute proteins, and we also found that tapeworms have a new subfamily of argonaute proteins (Supplementary Fig. 11.2) that may bind a newly discovered potential small RNA precursor[30]. Both *piwi* and *vasa* are usually essential in regulating the fate of germline stem cells in animals, and *vasa* suppression usually leads to infertility or death[31]. These findings suggest that stem-cell-associated pathways in parasitic flatworms may be highly modified.

## Specialization of the tapeworm proteome

We sought to identify novel and expanded gene families in tapeworms, and found many frequently occurring novel domains involved in cell–cell adhesion and the formation of the tegument (Supplementary Information, section 8). For example, several novel domains are found on the ectodomain of cadherins (Supplementary Information, section 8), and tapeworms have proportionally more tetraspanin copies (30–36) (Supplementary Table 12.1) than the highly expanded repertoires of fruitflies and zebrafish[32]. The acellular carbohydrate-rich laminated layer, which coats the outside of *Echinococcus* metacestodes, is a unique genus-specific trait and one of the few morphological traits that differ between the very closely related species *E. granulosus* and *E. multilocularis*. We identified corresponding species differences in an *Echinococcus*-specific apomucin family (Supplementary Fig. 12.1), an important building block of the laminated layer[33]. One particular copy is highly differentiated between the two species (non-synonymous to synonymous substitution ratio of >1) and is the fifth most highly expressed in the metacestode stage of *E. multilocularis* (Supplementary Table 5.7). Galactosyltransferases that probably decorate the apomucins with galactose residues, the predominant sugar of laminated layer glycans, are similarly diverged[33] (Supplementary Information, section 8). Approximately 20% of the genes are exclusive to tapeworms, and these include many highly expressed antigen families, such as antigen B, the glycosylphosphatidylinositol (GPI)-anchored protein GP50 (ref. 34), and the vaccine target EG95 (ref. 35) (Supplementary Table 12.4).

One of the most striking gene family expansions in the tapeworm genomes is the heat shock protein 70 (Hsp70) family. Phylogenetic analysis revealed independent and parallel expansions in both the Hsp110 and the cytosolic Hsp70 clades (Fig. 4). Several examples of expansions exist at various clades of Hsp70 in other systems, including Hsp110 expansions in oysters (to cope with temperature) and in cancer cells (to cope with proteotoxic stress)[36,37]. *Echinococcus* and *T. solium* have the highest number of gene expansions in the cytosolic Hsp70 clade. These expansions seem to have occurred independently in each species, and have resulted in 22 to 32 full copies in each species (*Echinococcus* and *T. solium*) compared to 6 copies in fruitflies and 2 in humans (Fig. 4). This expanded clade lacks classical cytosolic Hsp70 features (a conserved EEVD motif for substrate binding and a GGMP repeat unit), and whereas the canonical cytosolic *hsp70* genes are constitutively expressed in different life-cycle stages, the non-canonical genes show almost no expression, suggesting a putative

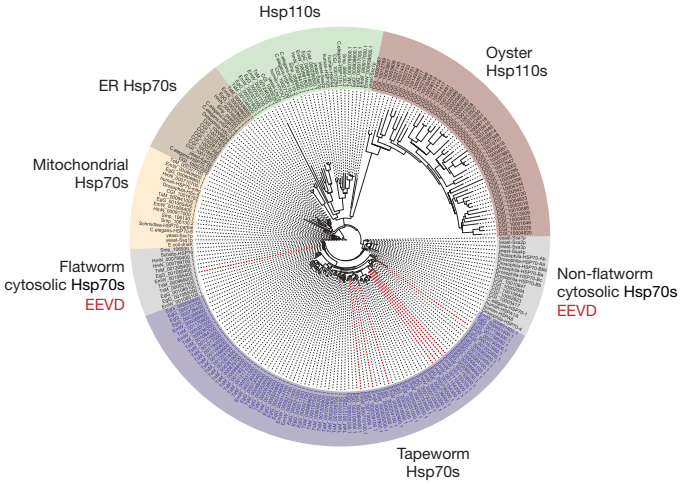



**Figure 4 | Heat shock protein 70 expansions in tapeworms.** Rooted tree of Hsp70 sequences from tapeworms and the eight comparator species used in this study, with additional sequences from baker's yeast *Saccharomyces cerevisiae*, and the Pacific oyster *Crassostrea gigas* (a non-flatworm example of a lophotrochozoan with a recently reported Hsp70 expansion). Different Hsp70 subfamilies are shown in different colours. Dotted red lines, *E. multilocularis hsp70* genes that are located in the subtelomeres. EEVD, the conserved carboxy-terminal residues of a canonical cytosolic Hsp70; ER Hsp70, endoplasmic reticulum Hsp70.

contingency role in which individual copies of the expanded family are only highly expressed under certain conditions (Supplementary Fig. 12.2). At least 40% of *E. multilocularis hsp70*-like genes are found within the subtelomeric regions of chromosomes, including the extreme case of chromosome 8 in which eight copies (including pseudogenes) are located in the subtelomere (Supplementary Table 12.2). No other genes are over-represented in these regions. Although Hsp70 proteins have been found in excretory–secretory products of tapeworms[38], it remains to be determined whether the non-canonical Hsps have a host-interacting role or whether telomere proximity is important for their function or expression.

## Novel drug targets

Tapeworm cysts are treated by chemotherapy or surgical intervention depending on tapeworm species, patient health and the site of the cyst. The only widely used drugs to treat tapeworm cysts are benzimidazoles[39] that, owing to considerable side effects, are administered at parasitistatic rather than parasiticidal concentrations[40]. Novel targets and compound classes are therefore urgently needed.

To identify new potential drug targets, we surveyed common targets of existing pharmaceuticals; kinases, proteases, G-protein-coupled receptors (GPCRs) and ion channels[41]. We identified approximately 250 to 300 new protein kinases (Supplementary Table 13.1), and these cover most major classes (Supplementary Information, section 13). We also identified 151 proteases and 63 peptidase-like proteins in *E. multilocularis*, a repertoire of similar diversity to *S. mansoni*, and found that, like *S. mansoni*, *E. multilocularis* has strongly reduced copy numbers compared to those of other animals (Supplementary Table 13.9). Many successful anthelminthic drugs target one of several different forms of neural communication[41]. We therefore mapped the signalling pathways of the serotonin and acetylcholine neurotransmitters, predicted conserved and novel neuropeptides (Supplementary Table 13.6), and classified more than 60 putative GPCRs (Supplementary Table 13.2) and 31 ligand-gated ion channels (Supplementary Table 13.4). A voltage-gated calcium channel subunit[42]—the proposed target of praziquantel—is not expressed in cysts and thus provides a putative explanation for the drug's low efficacy.

We searched databases for potential features for target selection, including compounds associated with protein targets and expression in the clinically relevant metacestode life-stage, and using this information we assigned weights to rank the entire proteomes (Supplementary Table 13.10). We identified 1,082 *E. multilocularis* proteins as potential targets, and of these, 150 to 200 with the highest scores have available chemical leads (known drug or approved compounds).

Acetylcholinesterases, which are inhibited by mefloquine (an antimalarial that reduces egg production in *S. mansoni*), are high on the list of potential targets[43]. However, acetylcholinesterase transcription in tapeworm cysts is low, possibly limiting their suitability. After filtering to remove targets with common substrates rather than inhibitors, the top of the list includes several homologues of targets for

**Table 1 | Top 20 promising targets in *E. multilocularis***

| Target category | Target | Action | Expression | Drug | Rank |
|---|---|---|---|---|---|
| Current targets | Tubulin β-chain | Cytoskeleton | M,A | Albendazole | 406 |
| | Voltage-dependent calcium channel | Ion transport | - | Praziquantel | 277 |
| Potential target | Thioredoxin glutathione reductase (TGR) | Detoxification | M,A | Experimental compounds | 277 |
| Top predicted targets | Fatty acid amide hydrolase | Bioactive lipid catabolism | M | Thiopental, propofol | 1 |
| | Adenine nucleotide translocator | Mitochondrial ATP export | M | Clodronate | 2 |
| | Inosine 5′ monophosphate dehydrogenase | Purine biosynthesis | M | Mycophenolic acid, ribavirin | 3 |
| | Succinate semialdehyde dehydrogenase | GABA catabolism | M | Chlormerodrin | 3 |
| | Ribonucleoside diphosphate reductase | Purine biosynthesis | M,A | Motexafin gadolinium | 5 |
| | Casein kinase II | Cell-cycle regulating kinase | M,A | Experimental compounds | 6 |
| | Hypoxanthine guanine phosphoribosyltransferase | Purine biosynthesis | M,A | Azathioprine | 8 |
| | Glycogen synthase kinase 3 | Multiple signalling pathways | M,A | Lithium | 8 |
| | Proteasome subunit | Protein degradation | M,A | Bortezomib | 16 |
| | Calmodulin | Transduces calcium signals | M,A | Trifluoperazine | 19 |
| | FK506 binding protein | Protein folding | M,A | Pimecrolimus | 19 |
| | UMP–CMP kinase | Phosphorylases ribonucleotides | M | Gemcitabine | 39 |
| | $Na^+/K^+$ ATPase | Ion transport | M | Artemether | 42 |
| | Carbonic anhydrase II | Acidity control | M | Multiple (for example, Methazolamide) | 42 |
| | NADH dehydrogenase subunit 1 | Energy metabolism | M | Multiple (for example, Methoxyflurane) | 42 |
| | Translocator protein | Multiple functions | M,A | Multiple (for example, Lorazepam) | 42 |
| | Elongation factor 2 | Translation | M,A | Experimental compounds | 54 |
| | Cathepsin B | Protease | M | Experimental compounds | 55 |
| | Dual-specificity mitogen activated protein | Signalling, activation of p38 | M | Experimental compounds | 56 |
| | Purine nucleoside phosphorylase | Purine metabolism | M,A | Didanosine | 63 |

A, adult; M, metacestode. Rank is sorted starting from the highest overall score; proteins with tied scores have the same rank. For current targets, the rank is only reported from the highest-scoring protein family member. For full scores and information please see Supplementary Table 13.10.

cancer chemotherapy, including casein kinase II, ribonucleoside reductase, UMP–CMP kinase and proteasome subunits (Table 1). The challenges of inhibiting cancer tumours and metacestodes (particularly those of *E. multilocularis*) with drugs are in some ways similar; both show uncontrolled proliferation, invasion and metastasis, and are difficult to kill without causing damage to the surrounding tissue. Therefore, metacestodes may be vulnerable to similar strategies as cancer; suppression of mitosis, induction of apoptosis and prevention of DNA replication. In fact, the anthelminthic medicines niclosamide, mebendazole and albendazole have already been shown to inhibit cancer growth[44].

## Conclusion

Tapeworms were among the first known parasites of humans, recorded by Hippocrates and Aristotle in ~300 BC (ref. 45), but a safe and efficient cure to larval tapeworm infection in humans has yet to be found. These genomes provide hundreds of potential drug targets that can be tested using high-throughput drug screenings that were made possible by recent advances in axenic and cell culturing techniques[39,46,47]. Flatworms display an unusually high degree of developmental plasticity. In this study, the high level of sequence completion enabled both gene losses and gains to be accurately determined, and has shown how this plasticity has been put to use in the evolution of tapeworms.

## METHODS SUMMARY

Genome sequencing was carried out using a combination of platforms. RNA sequencing was performed with Illumina RNA-seq protocols (for *E. multilocularis*, *E. granulosus* and *H. microstoma*) or capillary sequencing of full-length complementary DNA libraries (*T. solium*). The complete genome annotation is available at http://www.genedb.org. The tapeworm genome projects were registered under the INSDC project IDs PRJEB122 (*E. multilocularis*), PRJEB121 (*E. granulosus*), PRJEB124 (*H. microstoma*) and PRJNA16816 (*T. solium*). Sequence data for *T. solium* isolate (from Mexico) were used for all orthologue comparisons, but results relating to gene gains and losses were reconciled against an additional sequenced isolate from China (unpublished). All experiments involving jirds (laboratory host of *E. multilocularis*) were carried out in accordance with European and German regulations relating to the protection of animals. Ethical approval of the study was obtained from the ethics committee of the government of Lower Franconia (621-2531.01-2/05). Experiments with dogs (host of *E. multilocularis* sample RNA-seq ERS018054) were conducted according to the Swiss guidelines for animal experimentation and approved by the Cantonal Veterinary Office of Zurich prior to the start of the study, and were carried out with facility-born animals at the experimental units of the Vetsuisse Faculty in Zurich (permission numbers 40/2009 and 03/2010). A licensed hunter hunted the fox (host of *E. multilocularis* sample RNA-seq ERS018053) during the regular hunting season. *Hymenolepis* parasites were reared using laboratory mice in accordance with project license PPL 70/7150, granted to P.D.O. by the UK Home Office.

**Received 9 November 2012; accepted 21 February 2013.**
**Published online 13 March 2013.**

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**Supplementary Information** is available in the online version of the paper.

**Acknowledgements** We thank M. Dunn and OpGen for the *E. multilocularis* optical map; Roche for 20kb 454 libraries; R. Rance, M. Quail, D. Willey, N. Smerdon and K. Oliver for sequencing libraries at WTSI; T. D. Otto for bioinformatics expertise; R. Davies and Q. Lin for help with data release; A. Bateman, M. Punta, P. Cogghill and J. Minstry of Pfam; N. Rowlings from MEROPS database, J. Gough at SUPERFAMILY and C. Dessimoz for OMA; C. Seed and F. Jarero for laboratory assistance; J. Overington and B. Al-Lazikani; and B. Brejová for predicting genes with ExonHunter. P.D.O. and N.P.S. were supported in part by a BBSRC grant (BBG0038151) to P.D.O. P.D.O. and Ma.Z. were supported by a SynTax joint UK Research Council grant. The European Research Council supported P.W.H.H. and J.Paps. J.Parkinson and S.S.H. were supported by an operating grant from the Canadian Institute for Health Research (CIHR MOP#84556). G.S. was supported by FIRCA-NIH (grant TW008588) and the Universidad de la República, CSIC (grant CSIC 625). The *E. multilocularis*, *E. granulosus* and *H. microstoma* genome projects were funded by the Wellcome Trust through their core support of the Wellcome Trust Sanger Institute (grant 098051). K.B. was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG; BR2045/4-1). The *Taenia solium* Genome Project (IMPULSA 03) was supported by the Universidad Nacional Autonóma de México. The *Taenia solium* Genome Consortium thanks P. de la Torre, J. Yañez, P. Gaytán, S. Juárez and J. L. Fernández for technical support; L. Herrera-Estrella and LANGEBIO, CINVESTAV-Irapuato and C. B. Shoemaker for sequencing support and other advice; and J. Watanabe (deceased), S. Sugano and Y. Suzuki for the construction and sequencing of the full-length cDNA library.

**Author Contributions** I.J.T., Ma.Z., N.H. and M.B. Wrote the manuscript; M.B., K.B., J.P.L., X.S. and X.C. conceived and designed the project; N.H., M.B. and Ma.Z. coordinated the project. R.J.B., P.D., C.F., T.H., J.H., K.L., X.L., S.H., N.M., P.D.O., M.R., E.S., N.P.S., Y.Z. and K.B. prepared parasite material and nucleic acids; I.J.T., Ma.Z., A.G., K.E. and A.S.F. were involved in genome assembly; I.J.T., K.L.B., A.T., H.B., S.N., T.H., A.G. and K.E. were involved in genome assembly improvement; I.J.T., Ma.Z., A.S.F., X.S., K.L., J.H., A.G. and K.E. were involved in gene predictions; I.J.T., Ma.Z., K.L.B., A.T., H.B., O.L., S.N., R.C., R.J.B., G.F., E.S., X.S., J.P.L., K.L., J.H., A.G., K.E., S.H. and X.C. were involved in gene annotation; N.D.S., M.A., J.A.K., K.E., J.H., S.H., X.S. and Y.Z. were involved in data processing, computational and bioinformatics support; I.J.T. analysed genome structure, comparative genomics and ploidy; A.S.F., I.J.T. and Ma.Z. analysed gene structure; T.H. and H.M.B. experimentally validated micro-exons; A.G., K.B., F.K. and I.J.T. were involved with *trans*-splicing and polycistrons; I.J.T., S.S.H., J.Parkinson, G.S. and Ma.Z. examined metabolism and detoxification; P.W.H.H., J.Paps, N.R. and P.D.O. examined homeobox gene loss; K.B., I.J.T. and Ma.Z. examined stem cell specializations; Ma.Z. and J.D.W. examined domains; I.J.T., Ma.Z., C.F. and G.S. examined tapeworm-specific genes and expansions; Ma.Z. examined kinases and proteases; T.A.D. and Mo.Z. examined GPCRs; Mo.Z., T.A.D., U.K. and K.B. examined neuropeptides; M.R., L.K., F.C. and M.C. examined neuronal signalling; Ma.Z. examined drug targets; and G.S., M.R., C.F., K.B., P.W.H.H., P.D.O., A.G., R.J.B., G.F., E.S., X.S., J.P.L. and J.C. commented on the manuscript drafts.

**Author Information** The tapeworm genome projects were registered under the INSDC project IDs PRJEB122 (*E. multilocularis*), PRJEB121 (*E. granulosus*), PRJEB124 (*H. microstoma*) and PRJNA16816 (*T. solium*, Mexico). Illumina and 454 data are released to the European Nucleotide Archive (http://www.ebi.ac.uk/ena/) under accession numbers ERP000351, ERP000452 and PRJNA16816. Capillary data is at http://www.ncbi.nlm.nih.gov/Traces/trace.cgi, SEQ_LIB_ID 98488, 98489 and 101760, CENTER NAME SC (*E. multilocularis*) and sg1, sg2, sg3, sg4 and sg5 (*T. solium*, Mexico). Genome data are available from http://www.sanger.ac.uk/resources/downloads/helminths/ (*E. multilocularis*, *E. granulosus* and *H. microstoma*) and http://www.taeniasolium.unam.mx/taenia/ (*T. solium*). The complete genome annotation is available at http://www.genedb.org. All RNA-seq data were released to ArrayExpress under accession numbers E-ERAD-50 or E-ERAD-56. *T. solium* EST sequences were released to http://www.ncbi.nlm.nih.gov/nucest/, under accession numbers EL740221 to EL763490. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to M.B. (mb4@sanger.ac.uk), K.B. (kbrehm@hygiene.uni-wuerzburg.de) or J.P.L. (laclette@biomedicas.unam.mx).



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