Acta Tropica 148 (2015) 51–57

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Draft genome of neurotropic nematode parasite *Angiostrongylus cantonensis*, causative agent of human eosinophilic meningitis

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ARTICLE INFO

Article history:
Received 24 November 2014
Received in revised form 9 April 2015
Accepted 14 April 2015
Available online 21 April 2015

Keywords:
Angiostrongylus cantonensis
Genome
Kinases
Transferases
Excretory–secretory proteins
Metalloproteases
Homeobox genes
Neurotransmitters

ABSTRACT

Angiostrongylus cantonensis is a bursate nematode parasite that causes eosinophilic meningitis (or meningoencephalitis) in humans in many parts of the world. The genomic data from *A. cantonensis* will form a useful resource for comparative genomic and chemogenomic studies to aid the development of diagnostics and therapeutics. We have sequenced, assembled and annotated the genome of *A. cantonensis*. The genome size is estimated to be ~260 Mb, with 17,280 genomic scaffolds, 91X coverage, 81.45% for complete and 93.95% for partial score based on CEGMA analysis of genome completeness. The number of predicted genes of ≥300 bp was 17,482. A total of 7737 predicted protein-coding genes of ≥50 amino acids were identified in the assembled genome. Among the proteins of known function, kinases are the most abundant followed by transferases. The draft genome contains 34 excretory–secretory proteins (ES), a minimum of 44 Nematode Astacin (NAS) metalloproteases, 12 Homeobox (HOX) genes, and 30 neurotransmitters. The assembled genome size (260 Mb) is larger than those of *Pristionchus pacificus*, *Caenorhabditis elegans*, *Necator americanus*, *Caenorhabditis briggsae*, *Trichinella spiralis*, *Brugia malayi* and *Loa loa*, but smaller than *Haemonchus contortus* and *Ascaris suum*. The repeat content (25%) is similar to *H. contortus*. The GC content (41.17%) is lower compared to *P. pacificus* (42.7%) and *H. contortus* (43.1%) but higher compared to *C. briggsae* (37.69%), *A. suum* (37.9%) and *N. americanus* (40.2%) while the scaffold N50 is 42,191. This draft genome will facilitate the understanding of many unresolved issues on the parasite and the disorder it causes.

1. Introduction

Angiostrongylus cantonensis is an important emerging pathogen causing human eosinophilic meningitis (or meningoencephalitis) with thousands of cases in many parts of the world (Kliks and Palumbo, 1992; Wang et al., 2012). Presently, it has spread from its typical endemic regions of Asia and the Pacific to many other regions of the world, including the Americas, Australia, Caribbean islands and Africa (Eamsobhana, 2014). Its natural life cycle involves a definitive rodent host and a mollusk intermediate host. The adult worms live in the pulmonary arteries of rats. Humans are accidentally or incidentally infected with this parasite by ingestion of the third-stage larvae in intermediate hosts, paratenic hosts or contaminated raw or undercooked vegetables. The migration of larvae through the brain results in cerebral hemorrhage and eosinophilic meningitis, which can be fatal. Treatment for eosinophilic meningitis due to *A. cantonensis* is generally symptomatic and supportive in nature. Because of its importance in public health, *A. cantonensis* has received great attention in laboratory and clinical studies (Graeff-Teixeira et al., 2009). In particular, immunodiagnosis of human angiostrongyliasis has been explored extensively (Eamsobhana and Yong, 2009). Recently a number of studies have been reported on *A. cantonensis* high throughput sequencing (Morassutti et al., 2013a), microRNAs (Chang et al., 2013) and transcriptome profiling (Chang et al., 2013; Wang et al., 2013).

The DNA random high throughput sequencing based on 454 (Roche) platform by Morassutti et al. (2013a) consisted of 141 351

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http://dx.doi.org/10.1016/j.actatropica.2015.04.012
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contigs with an average length of 0·8 kb each. The putative ORFs was 28,080 of which 3371 had homology to other deposited protein sequences.

Here we report the draft genome of this food-borne zoonotic parasite, a member of the Metastrongyloidea superfamily of bursate nematodes and a *Wolbachia*-free nematode parasite. The genomic data from *A. cantonensis* will form a useful resource for comparative genomic and chemogenomic studies to aid the development of diagnostics and therapeutics.

2. Materials and methods

2.1. Biological material and DNA isolation

The rat lungworm *A. cantonensis* was from a laboratory strain originated in Thailand maintained in albino rat (*Rattus norvegicus*). 20 μg of DNA was extracted from a female adult worm using a DNeasy blood and tissue kit (Qiagen, Germany) and sequencing libraries with insert sizes of 300 bp, 500 bp, and 800 bp were constructed. After library preparation and quality control of DNA samples, template DNA fragments were hybridized to the surface of flow cells and amplified using REPLI-g Single Cell Kit (Qiagen, USA) to form clusters and subjected to paired-end sequencing.

2.2. Methods summary

The genome of *A. cantonensis* was sequenced using cluster generation and the sequencing was performed according to the cluster generation manual and sequencing manual from Illumina (Cluster Station User Guide and Genome Analyzer Operations Guide). In total, 24 Gb of DNA sequence data was generated (equal to 214-fold coverage of the whole genome) and used for de novo genome assembly. Base calls were generated using Casava 1.8.2 (Illumina), and the resulting de-multiplexed sequence reads were filtered for low quality. Sequence data from each library were sampled and low occurrence observations were filtered and assembly joins were optimized based on the available coverage and the kmer characteristics of each library. Assembly on the sequence data were performed using SOAPdenovo (Luo et al., 2012). AUGUSTUS (Stanke et al., 2004) and GlimmerHMM *ab initio* eukaryotic gene-finders (Majoros et al., 2004) were used for de novo gene prediction and CEGMA (Core Eukaryotic Genes Mapping Approach) (Parra et al., 2007) for validating the completeness of the assembly. Shared genes between *A. cantonensis* and 9 other nematode species (*Pristionchus pacificus*, *Caenorhabditis elegans*, *Necator americanus*, *Caenorhabditis briggsae*, *Trichinella spiralis*, *Brugia malayi*, *Loa loa*, *Haemonchus contortus* and *Ascaris suum*) were determined *via* psiblast. The results were filtered using three parameters, namely the *e*-value (*e*-value 1e − 5), amino acid length of ≥50 and sequence similarity of ≥40%. To detect synteny blocks, LASTZ was used to perform the pairwise whole-genome alignment. All proteins predicted from the gene set were annotated using INTERPRO database for conserved protein domains, pathways, and Gene Ontology (GO) annotation.

2.3. Sequencing and assembly (Fig. 1)

The flow of the genome assembly from the raw data is summarized in Fig. 1. A previously reported approach to estimate genome size was adopted (Li et al., 2010). A K-mer refers to an artificial sequence division of K nucleotides. If the length of each K-mer is K bp, a K-mer should be contained in a raw sequence read with L bp. The frequency of each K-mer can be calculated from the raw sequence reads. The frequencies of K-mer along the sequence depth gradient generally follow a Poisson distribution for a given dataset. Thus, the genome size G is calculated as G = K_num/K_depth, where

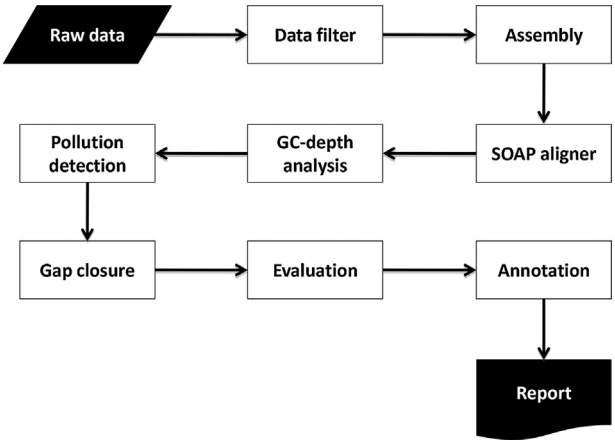

Fig. 1. Pipeline of genome assembly.

the K_num is the total number of K-mer, and K_depth is the sequencing depth that has the highest frequency of K-mer.

The filtered data was assembled using SOAPdenovo (Luo et al., 2012), a genome assembler specifically developed to assemble next-generation short read sequences (Li et al., 2010). It uses the de Bruijin algorithm (Pevzner et al., 2001) to assemble sequences from the Illumina reads, with a K-mer as a node and the K-1 bases overlapped between two K-mers as an edge. To reduce sequencing errors and limited branches, the tips and K-mers with low coverage in the graph were eliminated in the assembly process. The graph was transformed to a contig graph by transforming linearly connected K-mers into a pre-contig mode. Dijkstra's algorithm (Frana and Misa, 2010) was used to detect bubbles, which were merged into a single pathway when the branches sequences were identical. Using this method, repetitive sequences could be eliminated and consensus sequences obtained.

Contigs were linked to a scaffolding graph with paired-end (PE) reads. Sub-graph linearization was then applied to transform interleaving contigs into a linear structure and repeat masking was used to mask complicated connections for repeat contigs. Using this approach, contigs in any non-linear structure could be considered compatible. PE reads were applied subsequently step by step with increasing insert size of 300, 500 and 800 bp.

To fill gaps in the scaffolds, the PE reads were aligned and those that had one read well-aligned on a contig and another located in a gap region were retrieved, and a local assembly with the retrieved reads was performed.

3. Accession number

This Whole Genome Sequencing project has been deposited at GenBank under the accession PRJNA260338.

4. Results and discussion

4.1. Genome features

The assembled genome size (~260 Mb) of *A. cantonensis* is larger than other major nematodes (Table 1) including, *P. pacificus* (Dieterich et al., 2008), *C. elegans* (*C. elegans* Sequencing Consortium, 1998), *N. americanus* (Tang et al., 2014), *C. briggsae* (Stein et al., 2003), *T. spiralis* (Mitreva et al., 2011), *B. malayi* (Ghedin et al., 2007) and *L. loa* (Desjardins et al., 2013), but smaller than *H. contortus* (Schwarz et al., 2013) and *A. suum* (Jex et al., 2011).

The repeat content of *A. cantonensis* genome (25%, Table 2) is similar to *H. contortus*. The GC content (41.17%) is lower compared to *P. pacificus* (42.7%) and *H. contortus* (43.1%) but higher compared

Table 1
Summary of assembly statistics of major nematode genomes.

Statistics	Strategy	Genome size (Mb)	Scaffold N50	No. of scaffolds	Total length (bp)	Total length of gaps (bp)	GC content (%)	Reference
A. cantonensis	Illumina Hiseq	260	42,191	17,280	260,768,283	25,114,564	41.2	Present study
P. pacificus	Whole Genome Shotgun	169	737,446	5106	169,747,139	–	42.7	Dieterich et al. (2008)
C. elegans	Whole Genome Shotgun	97	17,493,793	7	100,269,912	–	36.0	*C. elegans* Sequencing Consortium (1998)
N. americanus	454	244	211,861	11,864	244,075,060	35,901,450	40.2	Tang et al. (2014)
C. briggsae	Whole Genome Shotgun	104	14,512,975	12	108,478,526	2993,053	37.4	Stein et al. (2003)
T. spiralis	ABI 3730	64	6373,445	6863	63,525,422	4983,936	34.0	Mitreva et al. (2011)
B. malayi	Whole Genome Shotgun	93.7	41,308	24,286	93,659,149	6531,325	30.2	Ghedin et al. (2007)
L. loa	454	91.4	13,750	14,332	91,373,458	3846,281	31.0	Desjardins et al. (2013)
H. contortus	Whole Genome Shotgun	320	99,131	12,915	319,640,208	23,800,995	42.2	Schwarz et al. (2013)
A. suum	Illumina HiSeq	309	413,062	12,988	269,559,681	6969,645	37.9	Jex et al. (2011)

to *C. briggsae* (37.69%), *A. suum* (37.9%) and *N. americanus* (40.2%) while the scaffold N50 is 42,191.

4.2. Phylogenetic relationship

There is no association between *Wolbachia*-free and *Wolbachia*-positive species. The bursate nematodes (*A. cantonensis*, *H. contortus* and *N. americanus*) are closely related to each other compared to non-bursate nematodes (Fig. 2a). There are two distinct clades of the component taxa of Class Chromadorea (more recently diverged nematodes): (1) Order Strongylida (*A. cantonensis*, *N. americanus*, *H. contortus*), Order Ascaridida (*A. suum*) and Order Diplogasterida (*P. pacificus*); and (2) Order Rhabditida (*C. elegans*, *C. briggsae*) and Order Spirurida (*B. malayi*, *L. loa*). Class Enoplea (ancestral diverged nematodes, represented by *T. spiralis*) is distinct from Chromadorea.

4.3. Gene prediction

Although *A. cantonensis* and *H. contortus* share 1492 more genes compared to *C. elegans* (Fig. 2b), *C. elegans* genome was used for functional annotation and comparison as the *H. contortus* genome is not well curated. Based on GlimmerHMM *ab initio* eukaryotic gene-finders (Majoros et al., 2004), the number of predicted genes of ≥300 bp was 17,482. This is within the range reported for other nematodes, *e.g.* 15,445 for *L. loa*, 17,846 for *B. malayi* and 26,846 for *C. elegans*. A total of 7737 protein-coding genes of ≥50 amino acids were identified in the assembled genome (Tables A1–A8, Appendix A: Supplementary data). All data provided here relates only to these 7737 genes and not to any of the shorter sequences.

Fig. 3 depicts the synteny analysis based on the mitochondrial genomes of *A. cantonensis*, *N. americanus* and *C. elegans*.

Of the entire predicted protein-coding genes, about two-third of the proteins identified in the *A. cantonensis* genome are of unknown function (Fig. 4). Among the proteins of known function, kinases are the most abundant followed by transferases.

Table 2
Basic statistics of the *A. cantonensis* draft genome.

Estimated genome size (Mbp)	**260**
Number of scaffolds	**17,280**
Total length (bp)	**260,768,283**
Mean scaffold length (bp)	**15,090**
N50 scaffold length (bp)	**42,191**
Predicted protein-coding genes (de-novo) ≥50 amino acid	**7737**
Repeat content	**25%**
Percent Ns	**9.96%**
GC content	**41.17%**

4.4. Wolbachia-free nematode parasites

In this study there was no evidence of the endosymbiont bacteria *Wolbachia* as suggested by Tsai et al. (2007). Our results support the absence of *Wolbachia* endobacteria in *A. cantonensis* reported by Foster et al. (2008). According to Foster et al. (2008) bioinformatic and phylogenetic analyses of the *Wolbachia* gene sequences reported by Tsai et al. (2007) indicated that they most likely resulted from contamination with DNA from arthropods and filarial nematodes. *Wolbachia* has also been found to be absent in *A. costaricensis* (Foster et al., 2008), three *Dracunculus* species (Foster et al., 2014) and *Setaria didgitata* (Voronina et al., 2015).

4.5. Excretory–secretory proteins

The draft genome contains 34 excretory–secretory proteins (ES), which can penetrate the defensive barriers of the host to avoid immunoresponse and are highly detectable at the very beginning of infection (Dzik, 2006). ES protein such as galectins, peroxiredoxin, protease and heat shock (HSP) plays an important role in regulating stress response in nematode.

A minimum of nine galectins were found in the genome of *A. cantonensis* using blastp (Table A9, Appendix A: Supplementary data), where their sequence identity was higher than 30%. Galectins have been reported previously to be present in *C. elegans* (Morassutti et al., 2013b). These galectins appear to be important for production, secretion, or stability. Galectins secreted by *A. cantonensis* were shown to be immunoreactive to antibodies present in serum from angiostrongyliasis patients (Morassutti and Graeff-Teixeira, 2012).

Peroxiredoxin (PRX) plays a central role in H_2O_2 detoxification (Morassutti et al., 2012). A minimum of three peroxiredoxin coding genes were identified (Table A10, Appendix A: Supplementary data). It has been suggested that the variation in pathogenicity between nematode species are due to differences in PRX structure and expression (Li et al., 2011).

Proteases are fundamental enzymes that enable the parasite to enter tissues and acquire nutrients (Morassutti et al., 2012). The infective third-stage larvae of *A. cantonensis* secrete a proteolytic enzyme that is thought to be essential for both larval penetration and development in their host. A minimum of 3 proteases were detected from our study (Table A11, Appendix A: Supplementary data).

Heat shock proteins (HSP) are a highly conserved group of proteins that function as chaperones, aid in protein folding and ATP binding. A minimum of 19 HSPs were identified from *A. cantonensis* assembly (Table A12, Appendix A: Supplementary data). HSPs are also known for their role in response to stress and have been detected in ES products of many parasitic nematodes including *A. cantonensis* (Morassutti et al., 2012).

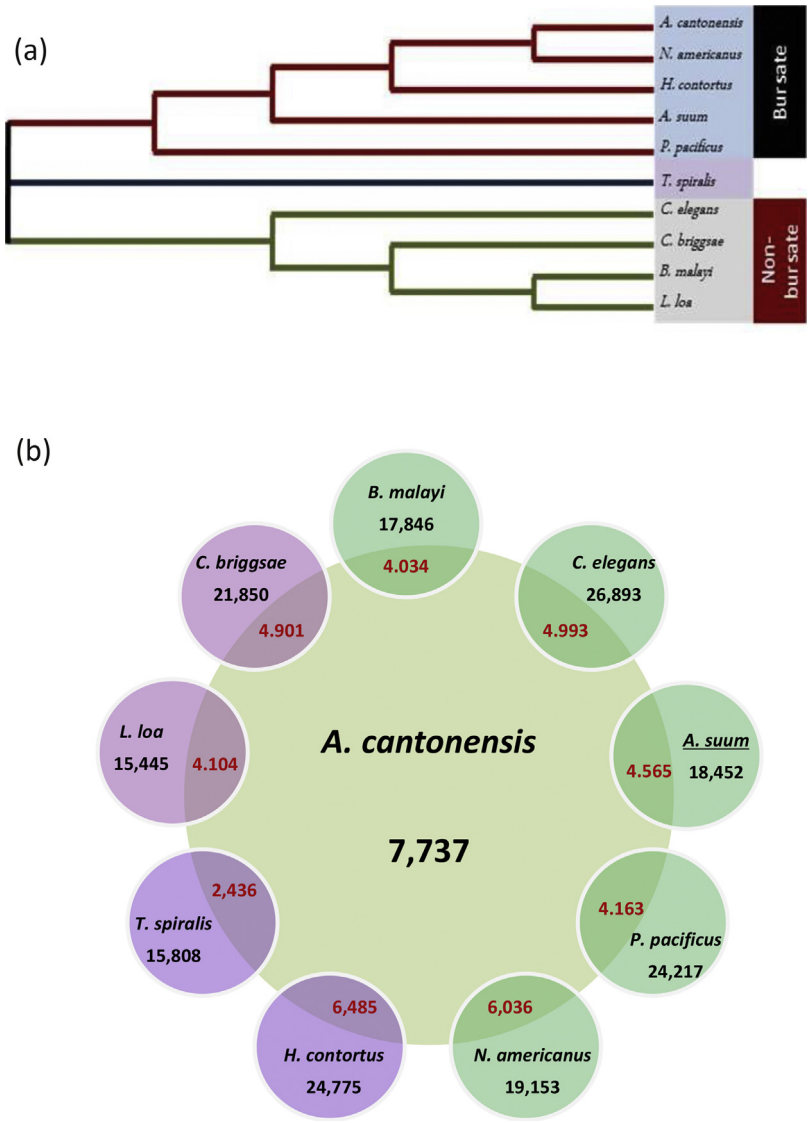

Fig. 2. Comparison of genomic features in major nematodes. (a) Schematic representation of the phylogenetic relationship of *A. cantonensis* in the context of nine other nematodes. All methods converged on a single topology with 100% support (either bootstrap values or posterior probabilities) at all nodes. (b) Venn diagram showing the number of shared protein-coding genes between *A. cantonensis* and other nematodes—figure for *A. cantonensis* refers to protein-coding genes of ≥50 amino acids, not toal number of predicted genes.

4.6. Nematode Astacin metalloproteases

In addition to ES proteins, a minimum of 44 Nematode Astacin (NAS) metalloproteases were encoded by the *A. cantonensis* genome (Table A13, Appendix A: Supplementary data). NAS has been linked to aid molting in nematodes. Defects in shedding of the cuticle were observed in worms lacking the NAS signifying a specific role of these enzymes that regulate the assembly of new cuticles by processing the precursors of extracellular matrix proteins (Stepek et al., 2009).

4.7. Homeobox genes

A minimum of 12 Homeobox (HOX) genes were identified from the sequence assembly (Table A14, Appendix A: Supplementary data). HOX genes are expressed along the body axis and link cells from different tissues to form a complex framework in nematodes (Teng et al., 2004). Auto-regulation, gene interactions and repression mechanisms regulated by HOX genes are common to many organisms (Teng et al., 2004).

4.8. Neurotransmitters

A minimum of 30 nerotransmitters were detected in *A. cantonensis* using INTERPRO database. Neuropeptides are the second category of neurotransmitters. They are small protein-like molecules localized to nervous tissue, but can be found in non-neuronal sites (McVeigh et al., 2008) which suggest that the function of some neuropeptides may vary. It was reported that neuropeptides encode secreted peptides with antibacterial activity and are up-regulated in response to bacterial challenge.

Expression of neuropeptide-like protein (NLP) NLP-29, 31 and 33 was found in microarray analyses in response to *C. elegans*, fungal or bacterial insults (Li and Kim, 2008). NLP-31 was expressed in the hypoderm and embryos and exhibited anti-microbial activity (Li and Kim, 2008). Interestingly, the fact that they were expressed in the hypoderm and have a defense response against bacteria could explain why *A. cantonensis* and *C. elegans* do not harbor the endosymbiont bacteria, *Wolbachia*. Within the filariid nematodes, *Wolbachia* is found in the hypodermal cells of the

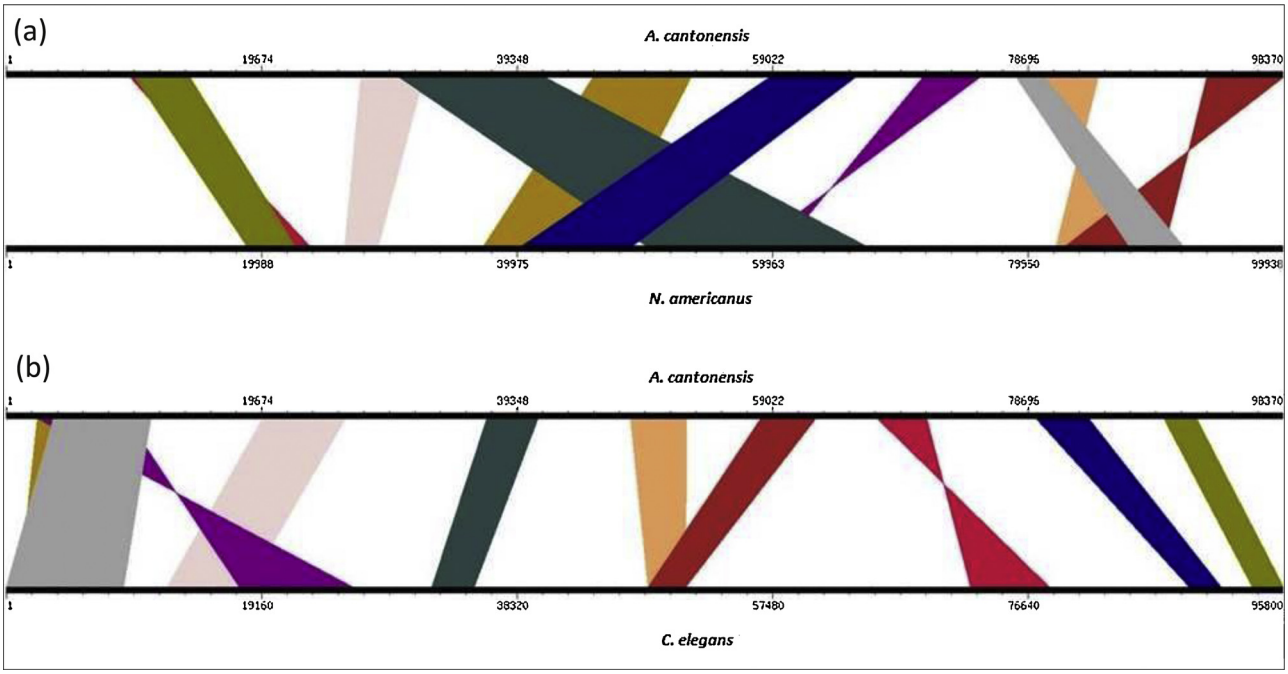

Fig. 3. Synteny analysis. (a) Comparison between *A. cantonensis* and *N. americanus* mitochondrial genome. (b) Comparison between *A. cantonensis* and *C. elegans* mitochondrial genome.

lateral chords of both sexes and in the ovaries, oocytes and developing intrauterine embryonic stages of female (Murfin et al., 2012).

Desjardins et al. (2013) suggested that *B. malayi*, which harbors *Wolbachia*, does not encode antibacterial peptides described in *C. elegans*, and that these molecules could either be dispensable in filariae or too divergent to be detected. However, the failure to detect NLP in other nematodes that lack *Wolbachia* (Table 3) could be explained by ancestral conditions or a secondary loss of the endosymbiont. Lateral gene transfer (LGT) could be another reason why these nematodes lack *Wolbachia*; their ancestors could have harbored the bacteria but eventually the genetic material from the bacteria was acquired by its nematode host, causing it to proliferate free from the endosymbiont. While ancestral condition and LGT could describe the absence of *Wolbachia* in the above nematode species (Table 3), the presence of antibacterial defensins molecules like neuropeptides, could provide an important insight into the absence of *Wolbachia* in *A. cantonensis*.

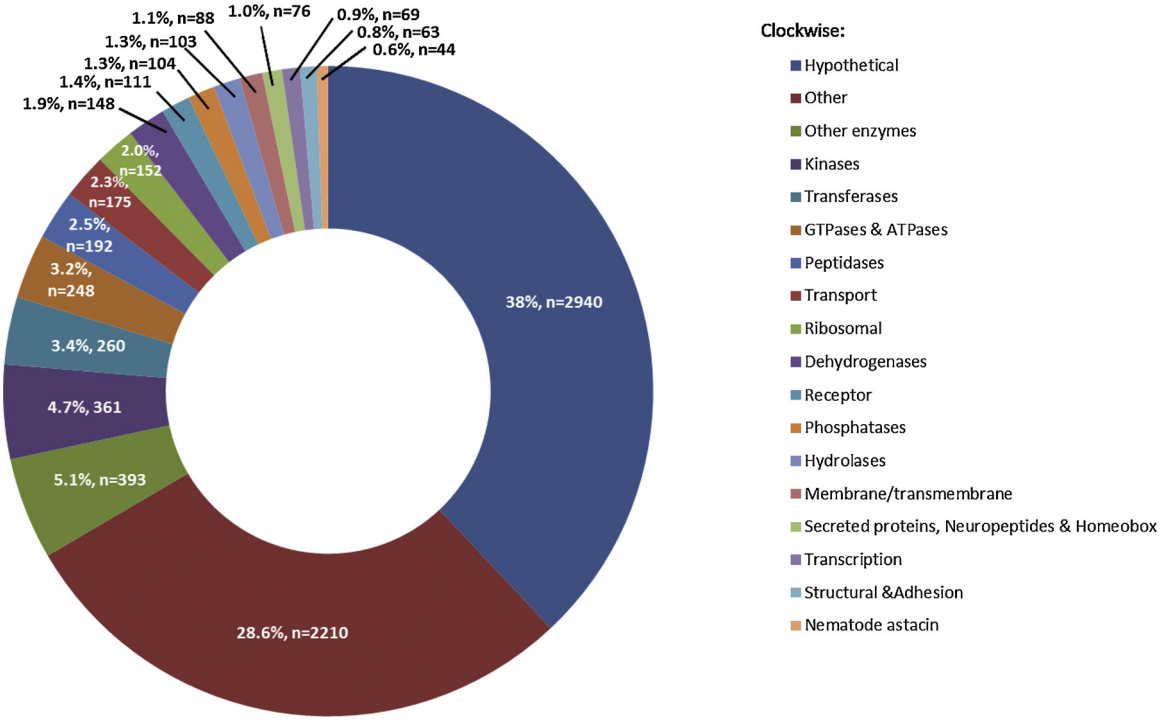

Fig. 4. Number of predicted protein classes by AUGUSTUS.

Table 3
Absence and presence of *Wolbachia* and its relation to NLP/CNC and other ES proteins.

Immune effector	*A. cantonensis*	*C. elegans*	*P. pacificus*	*B. malayi*	*A. suum*	*C. briggsae*	*H. contortus*	*L. loa*	*T. spiralis*	*N. americanus*
Antimicrobial caenacins (CNC)	3	11	0	0	0	0	0	0	0	0
Neuropeptide-like proteins (NLPs)	30	53	0	0	0	34	0	0	0	0
Galectin	9	14	0	8	0	9	0	8	10	0
Peroxiredoxin	3	35	0	7	0	12	0	2	4	3
NAS	44	46	0	20	0	40	0	38	28	0
HOX family	12	17	0	0	0	3	0	3	5	0
Presence of Wolbachia	No	No	No	Yes	No	No	No	No	No	No

Source: NCBI.

In conclusion, we have sequenced, assembled and annotated the genome of *A. cantonensis*, a metastrongyloid intra-arterial nematode that can cause inflammation of the brain and its meninges in human upon infection. Nearly 25 Gb of sequencing was carried out from 3 libraries (300 bp, 500 bp, and 800 bp) and the total number of reads was in excess of 235 million. The genome size of *A. cantonensis* is estimated to be 260 Mb, with 17,482 genes of ≥300 bp and 7737 protein-coding genes of ≥50 amino acids. Based on the filtered sequence data, the coverage is predicted to be 91×. CEGMA analysis indicates 93.15% (partial score) of genome completeness for the assembly. The number of contigs as well as the N50 for this genome assembly compares favorably with other published genomes. This is indication of the quality of the assembly produced. The present *A. cantonensis* draft genome should enable the identification of genes and other functional elements that are unique and common between related nematode species. This work should provide new insights into the genomics of *A. cantonensis* and attributes that are species-specific that may be related to adaptation to specific environments or hosts. It will facilitate the understanding of many unresolved issues on the parasite and the disorder it causes.

5. Addendum

After submission of our manuscript a wormbase genome announcement on the Taiwan isolate of *A. cantonensis* was available in GenBank (PRJEB493, ID: 261570 registered 20 September 2014). Our genome was deposited in GenBank on 11 September 2014 (BioProduct ID: PRJNA 260338). The Taiwan *A. cantonensis* genome was sequenced using 454 (Roche) platform and consisted of 18,635 scaffolds with an N50 of 43,900 bp and GC content of 41.54% compared to our genome based on Illumina HiSeq of 17,280 scaffolds with an N50 of 42,191 bp and GC content of 41.17%. The genome size of the Taiwan isolate is 253 Mb while ours is 260 Mb. CEGMA analysis based on 248 CEGs (core eukaryotic genes) shows identical partial score (93.95%) for both genomes. The complete score for the Taiwan isolate is 75.00% while that for our genome is 81.45%.

Acknowledgements

This study was funded in part by MoHE-HIR Grant (H-50001-00-A000025) and the University of Malaya (H-5620009). We thank our institutions for providing various research facilities and other support. We also thank the editor and three anonymous reviewers for their constructive comments and valuable suggestions which improved this paper.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actatropica.2015.04.012

References

C. elegans Sequencing Consortium, 1998. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. Science 282, 2012–2018.

Chang, S.H., Tang, P.P., Lai, C.H., Kuo, M.L., Wang, L.C., 2013. Identification and characterization of microRNAs in young adults of *Angiostrongylus cantonensis* via a deep-sequencing approach. Mem. Inst. Oswaldo Cruz 108, 699–706.

Desjardins, C.A., Cerqueira, G.C., Goldberg, J.M., Hotopp, J.C.D., Haas, B.J., Zucker, J., Ribeiro, J.M., Saif, S., Levin, J.Z., Fan, L., Zeng, Q., Russ, C., Wortman, J.R., Fink, D.L., Birren, B.W., Nutman, T.B., 2013. Genomics of *Loa loa*, a *Wolbachia*-free filarial parasite of humans. Nat. Genet. 45, 495–500.

Dieterich, C., Clifton, S.W., Schuster, L.N., Chinwalla, A., Delehaunty, K., Dinkelacker, I., Fulton, L., Fulton, R., Godfrey, J., Minx, P., Mitreva, M., Roeseler, W., Tian, H., Witte, H., Yang, S.P., Wilson, R.K., Sommer, R.J., 2008. The *Pristionchus pacificus* genome provides a unique perspective on nematode lifestyle and parasitism. Nat. Genet. 40, 1193–1198.

Dzik, J.M., 2006. Molecules released by helminth parasites involved in host colonization. Acta Biochim. Pol. 53, 33–64.

Eamsobhana, P., 2014. The Rat Lungworm *Angiostrongylus cantonensis*: Parasitology, Genetics and Molecular Phylogeny, second ed. Akson Graphic and Design Publishing House, Bangkok.

Eamsobhana, P., Yong, H.S., 2009. Immunological diagnosis of human angiostrongyliasis due to *Angiostrongylus cantonensis* (Nematoda: Angiostrongylidae). Int. J. Infect. Dis. 13, 425–431.

Foster, J.M., Kumar, S., Ford, L., Johnston, K.L., Ben, R., Graeff-Teixeira, C., Taylor, M.J., 2008. Absence of *Wolbachia* endobacteria in the non-filariid nematodes *Angiostrongylus cantonensis* and *A. costaricensis*. Parasit. Vectors 1, 31.

Foster, J.M., Landmann, F., Ford, L., Johnston, K.L., Elsasser, S.C., Schulte-Hostedde, A.I., Taylor, M.J., Slatko, B.E., 2014. Absence of *Wolbachia* endobacteria in the human parasitic nematode *Dracunculus medinensis* and two related *Dracunculus* species infecting wildlife. Parasit. Vectors 7, 140.

Frana, P.L., Misa, T.J., 2010. An interview with Edsgar W. Dijkstra. Commun. ACM 53, 41–47.

Ghedin, E., Wang, S., Spiro, D., Caler, E., Zhao, Q., Crabtree, J., Allen, J.E., Delcher, A.L., Guiliano, D.B., Miranda-Saavedra, D., Angiuoli, S.V., Creasy, T., Amedeo, P., Haas, B., El-Sayed, N.M., Wortman, J.R., Feldblyum, T., Tallon, L., Schatz, M., Shumway, M., Koo, H., Salzberg, S.L., Schobel, S., Pertea, M., Pop, M., White, O., Barton, G.J., Carlow, C.K., Crawford, M.J., Daub, J., 2007. Draft genome of the filarial nematode parasite *Brugia malayi*. Science 317, 1756–1760.

Graeff-Teixeira, C., da Silva, A.C.A., Yoshimura, K., 2009. Update on eosinophilic meningoencephalitis and its clinical relevance. Clin. Microbiol. Rev. 22, 322–348.

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., Vlaminck, J., Wang, T., Cantacessi, C., Schwarz, E.M., Ranganathan, S., Geldhof, P., Nejsum, P., Sternberg, P.W., Yang, H., Wang, J., Wang, J., Gasser, R.B., 2011. *Ascaris suum* draft genome. Nature 479, 529–533.

Kliks, M.M., Palumbo, N.E., 1992. Eosinophilic meningitis beyond the Pacific Basin: the global dispersal of a peridomestic zoonosis caused by *Angiostrongylus cantonensis*, the nematode lungworm of rats. Soc. Sci. Med. 34, 199–212.

Li, C., Kim, K., 2008. In: WormBook (Ed.), Neuropeptides. The *C. elegans* Research Community.

Li, R., Fan, W., Tian, G., Zhu, H., He, L., Cai, J., Huang, Q., Cai, Q., Li, B., Bai, Y., Zhang, Z., Zhang, Y., Wang, W., Li, J., Wei, F., Li, H., Jian, M., Li, J., Zhang, Z., Nielsen, R., Li, D., Gu, W., Yang, Z., Xuan, Z., Ryder, O.A., Leung, F.C., Zhou, Y., Cao, J., Sun, X., Fu, Y., Fang, X., Guo, X., Wang, B., Hou, R., Shen, F., Mu, B., Ni, P., Lin, R., Qian, W., Wang, G., Yu, C., Nie, W., Wang, J., Wu, Z., Liang, H., Min, J., Wu, Q., Cheng, S., Ruan, J., Wang, M., Shi, Z., Wen, M., Liu, B., Ren, X., Zheng, H., Dong, D., Cook, K., Shan, G., Zhang, H., Kosiol, C., Xie, X., Lu, Z., Zheng, H., Li, Y., Steiner, C.C., Lam, T.T., Lin, S., Zhang, Q., Li, G., Tian, J., Gong, T., Liu, H., Zhang, D., Fang, L., Ye, C., Zhang, J., Hu, W., Xu, A., Ren, Y., Zhang, G., Bruford, M.W., Li, Q., Ma, L., Guo, Y., An, N., Hu, Y., Zheng, Y., Shi, Y., Li, Z., Liu, Q., Chen, Y., Zhao, J., Qu, N., Zhao, S., Tian, F., Wang, X., Wang, H., Xu, L., Liu, X., Vinar, T., Wang, Y., Lam, T.W., Yiu, S.M., Liu, S., Zhang, H., Li, D., Huang, Y., Wang, X., Yang, G., Jiang, Z., Wang, J., Qin, N., Li, L., Li, J., Bolund, L., Kristiansen, K., Wong, G.K., Olson, M., Zhang, X., Li, S., Yang, H., Wang, J., Wang, J., 2010. The sequence and de novo assembly of the giant panda genome. Nature 463, 311–317.

Li, Z., Liu, X., Chu, Y., Wang, Y., Zhang, Q., Zhou, X., 2011. Cloning and characterization of a 2-Cys peroxiredoxin in the pine wood nematode. Bursaphelenchus xylophilus, a putative genetic factor facilitating the infestation. Int. J. Biol. Sci. 7, 823–836.

Luo, R., Liu, B., Xie, Y., Li, Z., Huang, W., Yuan, J., He, G., Chen, Y., Pan, Q., Liu, Y., Tang, J., Wu, G., Zhang, H., Shi, Y., Liu, Y., Yu, C., Wang, B., Lu, Y., Han, C., Cheung, D.W., Yiu, S.M., Peng, S., Xiaoqian, Z., Liu, G., Liao, X., Li, Y., Yang, H., Wang, J., Lam, T.W., Wang, J., 2012. SOAPdenovo2: an empirically improved memory-efficient short-read *de novo* assembler. Gigascience 1, 18.

Majoros, W.H., Pertea, M., Salzburg, S.L., 2004. TigrScan and GlimmerHMM: two open source *ab initio* eukaryotic gene-finders. Bioinformatics 20, 2878–2879.

McVeigh, P., Alexander-Bowman, S., Veal, E., Mousley, A., Marks, N.J., Maule, A.G., 2008. Neuropeptide-like protein diversity in phylum Nematoda. Int. J. Parasitol. 38, 1493–1503.

Mitreva, M., Jasmer, D.P., Zarlenga, D.S., Wang, Z., Abubucker, S., Martin, J., Taylor, C.M., Yin, Y., Fulton, L., Minx, P., Yang, S.P., Warren, W.C., Fulton, R.S., Bhonagiri, V., Zhang, X., Hallsworth-Pepin, K., Clifton, S.W., McCarter, J.P., Appleton, J., Mardis, E.R., Wilson, R.K., 2011. The draft genome of the parasitic nematode *Trichinella spiralis*. Nat. Genet. 43, 228–235.

Morassutti, A.L., Perelygin, A., De Carvalho, M.O., Lemos, L.N., Pinto, P.M., Frace, M., Wilkins, P.P., Graeff-Teixeira, C., Da Silva, A.J., 2013a. High throughput sequencing of the *Angiostrongylus cantonensis* genome: a parasite spreading worldwide. Parasitology 140, 1304–1309.

Morassutti, A.L., Perelygin, A., Levert, K., Lin, S.C., Lee, Y.M., da Silva, A.J., Wilkins, P.P., Graeff-Teixeira, C., 2013b. Expression of recombinant antigenic proteins from *Angiostrongylus cantonensis*: a brief report. Hawaii J. Med. Public Health 72 (6 Suppl. 2), 58–62.

Morassutti, A.L., Graeff-Teixeira, C., 2012. Interface molecules of *Angiostrongylus cantonensis*: their role in parasite survival and modulation of host defenses. Int. J. Inflamm., http://dx.doi.org/10.1155/2012/512097

Morassutti, A.L., Levert, K., Pinto, P.M., da Silva, A.J., Wilkins, P., Graeff-Teixeira, C., 2012. Characterization of *Angiostrongylus cantonensis* excretory–secretory proteins as potential diagnostic targets. Exp. Parasitol. 130, 26–31.

Murfin, K.E., Dillman, A.R., Foster, J.M., Bulgheresi, S., Slatko, B.E., Sternberg, P.W., Goodrich-Blair, H., 2012. Nematode–bacterium symbioses—cooperation and conflict revealed in the Omics age. Biol. Bull. 223, 85–102.

Parra, G., Bradnam, K., Korf, I., 2007. CEGMA: a pipeline to accurately annotate core genes in eukaryotic genomes. Bioinformatics 23, 1061–1067.

Pevzner, P.A., Tang, H., Waterman, M.S., 2001. An Eulerian path approach to DNA fragment assembly. Proc. Natl. Acad. Sci. U.S.A. 98, 9748–9753.

Schwarz, E.M., Korhonen, P.K., Campbell, B.E., Young, N.D., Jex, A.R., Jabbar, A., Hall, R.S., Mondal, A., Howe, A.C., Pell, J., Hofmann, A., Boag, P.R., Zhu, X.Q., Gregory, T., Loukas, A., Williams, B.A., Antoshechkin, I., Brown, C., Sternberg, P.W., Gasser, R.B., 2013. The genome and developmental transcriptome of the strongylid nematode *Haemonchus contortus*. Genome Biol. 14, R89, http://dx.doi.org/10.1186/gb-2013-14-8-r89

Stanke, M., Steinkamp, R., Waack, S., Morgenstern, B., 2004. AUGUSTUS: a web server for gene finding in eukaryotes. Nucl. Acids Res. 32, W309–W312, http://dx.doi.org/10.1093/nar/gkh379

Stein, L.D., Bao, Z.R., Blasiar, D., Blumenthal, T., Brent, M.R., Chen, N.S., Chinwalla, A., Clarke, L., Clee, C., Coghlan, A., Coulson, A., D'Eustachio, P., Fitch, D.H.A., Fulton, L.A., Fulton, R.E., Griffiths-Jones, S., Harris, T.W., Hillier, L.W., Kamath, R., Kuwabara, P.E., Mardis, E.R., Marra, M.A., Miner, T.L., Minx, P., Mullikin, J.C., Plumb, R.W., Rogers, J., Schein, J.E., Sohrmann, M., Spieth, J., Stajich, J.E., Wei, C.C., Willey, D., Wilson, R.K., Durbin, R., Waterston, R.H., 2003. The genome sequence of *Caenorhabditis briggsae*: a platform for comparative genomics. PLoS Biol. 1, 166–192.

Stepek, G., McCormack, G., Page, A.P., 2009. Collagen processing and cuticle formation is catalysed by the astacin metalloprotease DPY-31 in free-living and parasitic nematodes. Int. J. Parasitol. 40, 533–542.

Tang, Y.T., Gao, X., Rosa, B.A., Abubucker, S., Hallsworth-Pepin, K., Hotez, P.J., Bethony, J.M., 2014. Genome of the human hookworm *Necator americanus*. Nat. Genet. 46, 261–269.

Teng, Y., Girard, L., Ferreira, H.B., Sternberg, P.W., Emmons, S.W., 2004. Dissection of *cis*-regulatory elements in the *C. elegans* Hox gene egl-5 promoter. Dev. Biol. 276, 476–492.

Tsai, K.H., Huang, C.G., Wang, L.C., Yu, Y.W., Wu, W.J., Chen, W.J., 2007. Molecular evidence for the endosymbiont *Wolbachia* in a non-filaroid nematode, *Angiostrongylus cantonensis*. J. Biomed. Sci. 14, 607–615.

Voronina, D., Abeykoonc, A.M.L.L., Gunawardene, Y.I.S., Dassanayake, R.S., 2015. Absence of *Wolbachia* endobacteria in Sri Lankan isolates of the nematode parasite of animals *Setaria digitata*. Vet. Parasitol. 207, 350–354.

Wang, L.C., Chen, K.Y., Chang, H.S., Chung, L.Y., Gan, R.C., Cheng, C.J., Petrus, C., Tang, P., 2013. Trancriptome profiling of the fifth-stage larvae of *Angiostrongylus cantonensis* by next-generation sequencing. Parasitol. Res. 112, 3193–3202.

Wang, Q.P., Wu, Z.-D., Wei, J., Owen, R.L., Lun, Z.-R., 2012. Human *Angiostrongylus cantonensis*: an update. Eur. J. Clin. Microbiol. Infect. Dis. 31, 389–395.