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CattleTickBase: An integrated Internet-based bioinformatics resource for *Rhipicephalus (Boophilus) microplus* ★

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Note: Supplementary data associated with this article.
ABSTRACT

The *Rhipicephalus microplus* genome is large and complex in structure, making it difficult to assemble a genome sequence and costly to resource the required bioinformatics. In light of this, a consortium of international collaborators was formed to pool resources to begin sequencing this genome. We have acquired and assembled genomic DNA into contigs that represent over 1.8 Gigabase pairs (Gbp) of DNA from gene-enriched regions of the *R. microplus* genome. We also have several datasets containing transcript sequences from a number of gene expression experiments conducted by the consortium. A web-based resource was developed to enable the scientific community to access our datasets and conduct analysis through a web-based bioinformatics environment called YABI. The collective bioinformatics resource is termed CattleTickBase. Our consortium has acquired genomic and transcriptomic sequence data at approximately 0.9X coverage of the gene-coding regions of the *R. microplus* genome. The YABI tool will facilitate access and manipulation of cattle tick genome sequence data as the genome sequencing of *R. microplus* proceeds. During this process the CattleTickBase resource will continue to be updated.

Keywords: Cattle tick; Transcriptome; Genome project; Bioinformatics
1. Introduction

The global cattle population is estimated at approximately 1 billion. Of this population, 80% inhabit areas that have been considered suitable habitat for ticks and tick-borne diseases (Snelson, 1975). The cattle tick *Rhipicephalus (Boophilus) microplus* is considered the most significant cattle parasite in the world, having established populations in most of the world’s tropical and subtropical countries. This tick causes blood loss and physical damage to hides of infested animals. In addition, *R. microplus* is the vector for several bovine diseases, including babesiosis (caused by protozoan species *Babesia bovis* and *Babesia bigemina*) and anaplasmosis (caused by the rickettsia *Anaplasma marginale*), with severe impact on agricultural systems globally (de Castro, 1997). Economic losses to cattle producers from ticks and tick-borne diseases are US$13-18 billion globally on an annual basis (de Castro, 1997). Annual losses attributable to *R. microplus* in Brazil and Australia alone are estimated at US$2 billion (Grisi et al., 2002) and AUS$175 million (Playford et al., 2005), respectively.

Ticks are believed to be among the most ancient terrestrial arachnids and possibly the earliest organisms to have evolved blood-feeding capabilities (Mans and Neitz, 2004). *Rhipicephalus microplus* is a single-host species and has evolved such that it must maintain sustained contact with its host during the life stages, from the attached and feeding larva through to the fully engorged female. This period of attachment typically lasts approximately 3 weeks with some variation depending on environmental conditions. The species has developed a unique means of avoiding the host animal’s immune responses during infestation (Wikl, 1999) and *R. microplus* salivary gland extracts have been shown to have an immunosuppressive effect on the bovine host (Turni et al., 2004, 2007). The tick must also respond to many
microorganisms, both symbiotic and parasitic, from the external environment or those ingested through feeding-associated activities (Andreotti et al., 2011).

With this interplay between bovine host, tick and microbiota, determining the whole genome sequence of *R. microplus* will greatly advance tick gene discovery, enable a better understanding of tick-host-pathogen immunology and provide insight on how the cattle tick responds to environmental perturbations, including pressures from moisture and temperature extremes and acaricidal applications. The *R. microplus* genome size is estimated to be 7.1 Gigabase pairs (Gbp), more than twice the size of the human genome, and consists of greater than 70% repetitive DNA (Ullmann et al., 2005). It is therefore a challenge for de novo assembly, even with contemporary DNA sequencing technologies. A 4X shotgun coverage genome sequence for the blacklegged tick, *Ixodes scapularis*, is available (Lawson et al., 2009) and is the only reported tick genome sequence to date. The version 1.1 sequence assembly consists of 369,492 supercontigs, totalling 1.76 Gbp with a supercontig N50 size of 72kb.

From a taxonomic perspective, both *R. microplus* and *I. scapularis* are classified as hard ticks. There are two lineages of hard ticks, the Prostriata, which consists of the single genus *Ixodes* containing approximately 250 species, and the Metastriata, which consists of approximately 464 species from several genera including *R. microplus* (Barker and Murrell, 2004). Given the sequence divergence between *R. microplus* and *I. scapularis* (Guerrero et al., 2006), gene discovery efforts solely using *I. scapularis* as the model tick genome would prove limiting for *R. microplus* research efforts.

Towards a goal of generating a genetic resource for this economically important tick species, efforts have focused on a combination of sequencing
strategies. The goal of this project was to maximize the utility of the data that could be generated with the resources available. To date, these strategies include Cot-filtered genomic DNA sequencing, bacterial artificial chromosome (BAC)-end sequencing (BES), targeted whole BAC sequencing, whole transcriptome sequencing and small RNA sequencing. Presently, we have acquired, assembled and annotated over 2 Gb of sequence data. This is comprised of 1.7 Gb of assembled contigs from three Cot reassociation experiments. These Cot experiments utilised methodologies to select randomly sheared genomic DNA for fractions depleted in highly repetitive sequences and enriched for putative gene coding regions (Guerrero et al., 2010). Also available are three transcriptome library assemblies (21 Mb) representing over 33,000 transcripts. Integrating data generated from these various approaches is already starting to provide new insights into the very large and complex cattle tick genome.

This paper provides an overview of the coordinated cattle tick genome sequence resource as well as a new internet-based bioinformatics resource that is designed to integrate our various genomic and transcriptomic datasets. This enables the cattle tick research community to access and analyse genomic and transcriptomic data at a single on-line resource, similar to approaches used by insect and worm researchers e.g. FlyBase, WormBASE (Harris and Stein, 2006; Drysdale, 2008). Examples are provided of these new genomic analysis tools and how they can be utilised by the research community to understand the genomic structure, organisation and content of the cattle tick genome.
2. Materials and methods

2.1. Source of tick materials

For the USA ticks, genomic and Cot DNA were extracted from eggs of the *R. microplus* Deutsch strain, f7, f10, f11 and f12 generations. These were pooled and a total of 10 g was used to purify very high molecular weight genomic DNA (Guerrero et al., 2010). This strain was started from only a few individual engorged females collected from a 2001 tick outbreak in South Texas. Although the strain has been inbred since its creation in 2001, it is not genetically homogeneous. For the Australian ticks, the larvae and fully engorged N strain of Australian *R. microplus* were utilized in these analyses. The N strain is maintained by the Biosecurity laboratories at the Department of Employment, Economic Development and Innovation (DEEDI), Queensland, under controlled conditions of 28 °C and 80% relative humidity prior to bovine infestation (Stewart et al., 1982).

2.2. Sequencing and assembly

For the BAC library synthesis, approximately 2 g of larvae from the f8 generation of the Deutsch strain were used by Amplicon Express Inc. (Pullman, WA, USA) to isolate genomic DNA partially digested with *Mbo*I to synthesize a BAC library of approximately 0.8X coverage (Guerrero et al., 2010). Subsequently, a second library of 2.4X coverage was synthesized from genomic DNA partially digested with *Hind*III. Five BAC assemblies, BM-074-Random-F12, BM-077-Random-J09, BM-129-CzEst9-N14, BM-066-M07, BM-077-G20, are as described by Guerrero et al. (2010). The remaining 10 BAC sequences were trimmed for vector and bacterial contamination by phred-phrap software (Ewing and Green, 1998)
cross_match, with options set at minmatch 12 and minscore 20. Contig order and orientation were based on Phrapview paired end reads.

Total R. microplus genomic DNA was prepared and processed by three Cot filtration experiments to enrich for single/low-copy and moderately repetitive DNAs. Cot-filtered DNA was sequenced using 454 FLX and Titanium pyrosequencing (Research and Testing Laboratory, Lubbock, TX, USA). Methods are as described in Guerrero et al. (2010).

The filtered genomic DNA (total number of reads 7,289,230 and total number of bases 1,798,400,445) was de novo assembled using the Newbler assembler for 454 reads (Margulies et al., 2005) with default settings. All contigs (745,975 sequences) and BAC end sequences (BES) (GenBank Accession Numbers HN108288-HN118367) were then assembled with Cap3 (Huang and Madan, 1999) default settings. Whole Genome Shotgun (WGS) project ADMZ02000000 is the result of this two-step assembly.

2.3. BAC and Cot read alignment

BAC and Cot read alignments were carried out with BWA-SW (Li and Durbin, 2010) for long reads as our average read length was 245 bp. A mapping accuracy >99% was expected with a mapping quality (MapQ) of 10 and sensitivity (Z) of 100.

2.4. Gene predictions
Gene predictions for the BAC sequences and WGS were made with GenScan version 1.0 (Burge and Karlin, 1997) for default ‘optimal exons’, parameters for human/vertebrates and coding sequences (CDS) option. BAC predicted gene and AutoFACT (Koski et al., 2005) annotation can be found in Supplementary Table S1 and Supplementary Fig. S1.

2.5. Repeat analysis


2.6. RNA Searches: tRNA and rRNA

Transfer RNA (tRNA) searches were conducted with trnascan version 1.23 (Schattner et al., 2005) and rRNA using rnammer version 1.2 (Lagesen et al., 2007).

2.7. Sequence comparative analysis

The genomic data set Ixodes scapularis SUPERCONTIGS-Wikel.IscaW1.fa.gz (dataset downloaded from VectorBase (Lawson et al., 2009) Date: Sept. 21, 2010) was aligned to R. microplus assembled Cot DNA (GenBank WGS division second version, ADMZ02000000) using BLASTn (Altschul et al., 1990). Homologous regions of interest were selected at an expected value < 1e-50.
The *R. microplus* BAC sequences submitted to GenBank: **HM748958-HM748967** and five BACs as described in Guerrero et al. (2010) were aligned to the BES submitted to the Genome Survey Sequence (GSS) division of GenBank: **HN108288-HN118367** using BLAT with 70% identity, a length greater than 100 bp, and the option ‘fastMap’.

The *R. microplus* BES, predicted BAC gene content and new transcript sequences were comparatively aligned to Dana Farber Cancer Institute (DFCI) gene indices, IsGI version 3.0 and *Bmi*GI version 2.1 (Quackenbush et al., 2001), NCBI RefSeq mRNA, and the Subtraction Library clones as described previously (Lew-Tabor et al., 2009) using BLASTn at an expected value <1e-10, and to NCBI RefSeq Protein and GenPeptide, *iscapularis*.PEPTIDES-IscaW1.1.fa (VectorBase Date: Sep 21, 2010, Lawson et al., 2009) using BLASTx at an expected value <1e-10. The collective *R. microplus* transcriptome (*Rmi*Tr Version 1.0, Table 1) and *I. scapularis* (DFCI IscGI) sequences were searched using tBLASTx at an expected value of 1e-05 to uniref100, and orthologous sequences were determined for those alignments that had greater than or equal to 60% *R. microplus* sequence coverage and an amino acid conservation greater than or equal to 30%. Transcript alignments were also made using BLAT (Kent, 2002) to a comparative species with 70% identity and a length greater than 100 bp.

### 2.8. Transcriptome sequencing

For transcriptome sequencing, female adult dissected gut and ‘frustrated’ larvae were prepared as described previously from Australian N strain ticks (Lew-Tabor et al., 2009). The frustrated larval sample contains larvae placed in a gas-
permeable bag taped directly to the host animal feed source. Thus, the larvae are able
to sense the presence of the host but the bag presents a barrier that prevents
attachment and feeding. Approximately 30 mg of total RNA from each of these
samples were collected for high-throughput sequencing of the tick transcriptome
using the Illumina/GA single-end reads format as described previously (Mortazavi et
al., 2008).

2.9. Transcriptome assembly and clustering

The de novo transcriptome assembly of the adult female gut and frustrated
larvae transcriptomes using 60 bp single-end Illumina/GA reads was conducted using
Abyss (Birol et al., 2009) with k-mer sizes ranging from 36 to 64. The assembled
contigs were then clustered using cap3 (Huang and Madan, 1999) with a 98%
sequence identity threshold and an overlap region of at least 30 bases to remove
transcript redundancy. Non-redundant sets of transcripts for the two libraries can be
found on the CattleTickBase website.

The RmiTr version 1.0 data set contains sequences from DFCI_bmigi.V2.1,
adult female gut transcriptome, frustrated larvae transcriptome and R. microplus
subtraction library (Lew-Tabor et al., 2009), which were clustered into contigs using
cap3 (Huang and Madan, 1999) with following the options: -p 99.99999 -m 1 -n -10
-g 1 -b 16 -y 6.

2.10. YABI
The YABI application consists of a front-end web application responsible for
the user interface. Users create a secure account and are free to access the datasets and
analysis tools available within the system. Users can create bioinformatics pipelines
from the available tools. The tools currently available for sequence analysis include:
similarity/homology searches, feature prediction, high throughput downstream
analysis, assembly and annotation. Datasets (including other tick-related GenBank
bioprojects) in YABI are updated from GenBank/EMBL/DDBJ and VectorBase at
regular intervals. Individual dataset contributors can deposit and update data sets by
contacting yabi@ccg.murdoch.edu.au with an option to have a secure account to
conduct their analysis within their own teams. The Centre for Comparative Genomics
(CCG), Murdoch University, Australia is committed to supporting the bioinformatics
aspects of the *R. microplus* project. The CCG houses a supercomputer (currently
ranked number 87 in the world - [http://www.top500.org/list/2010/11/100](http://www.top500.org/list/2010/11/100)) and
provides support to national and international bioinformatics and other high-end
science-based activities. The CCG develops and deploys sophisticated software
solutions, supports and conducts a diverse range of bioinformatics analysis. Requests
and suggestions can be made by contacting info@ccg.murdoch.edu.au. The *R.
*microplus* datasets currently available for sequence similarity searching and other
bioinformatics analyses are summarised in Table 1.

3. Results

3.1. *Rhipicephalus microplus* datasets currently available

The nine datasets that are available for access and further analysis on the
CattleTickBase website are summarised in Table 1 and described below. For Dataset
1, total *R. microplus* genomic DNA was prepared and processed by three Cot filtration experiments to enrich for single/low-copy and moderately repetitive DNA. It was anticipated that the DNA obtained via this process would remove a significant portion of highly repetitive DNA and contain predominantly gene rich regions. The resulting DNA fragments ranged in size from 250 to 600 bp and were sequenced in three separate sequencing experiments with one experiment using six runs of 454 FLX pyrosequencing (Guerrero et al., 2010) and two experiments each using three Titanium 454 runs. The data from the latter two experiments have been deposited in GenBank SRA, submission: SRA012677.4/SID000001. Approximately 1.8 Gbp of sequence were generated and assembled. This assembly (Dataset 2) was submitted to GenBank Whole Genome Sequencing Project; under the United States Department of Agriculture, Agricultural Research Service (USDA-ARS) *R. microplus* Project ID 46685 assigned Project accession ADMZ0000000. The most recent version for this project reported in this paper has the accession number ADMZ02000000; this submission consists of 175,208 contig sequences of average contig size 825 bp and a maximum contig length of 9,681 bp. These sequences have been submitted under GenBank Accession Numbers ADMZ02000001-ADMZ02175208. Based on the estimate that the *R. microplus* genome size is approximately 7.1 Gbp, the assembled Cot DNA dataset represents approximately 2% of the *R. microplus* genome. We undertook a comparative analysis of this Cot DNA dataset with the BmiGI Version 2.1 Gene Index (http://compbio.dfci.harvard.edu/cgi-bin/tgi/gimain.pl?gudb=b_microplus; Quackenbush et al., 2001; Guerrero et al., 2005) to confirm that the Cot DNA filtration process filtered for gene rich regions. When we aligned the 175,208 Cot DNA contigs to the 14,586 BmiGI transcript contigs, 52% of the BmiGI entries had a match (>85% identity) to at least one contig in the Cot
dataset. Thus, even though the coverage of the Cot DNA over the entire 7.1 Gbp *R. microplus* genome was very low (2%), the coverage of the *BmiGI* transcriptome dataset was high (52%). This also indicates that deeper sequencing of the Cot-selected DNA would be warranted to obtain fuller coverage of the gene-encoding regions, as 48% of the *BmiGI* entries did not have a match in the Cot DNA dataset.

For Dataset 3, a 3.2x coverage BAC library was used to sequence 10,582 BES resulting in 7,290,530 bp of sequence. The amount of BES data generated represents approximately 0.1% of the *R. microplus* genome. BES greater than 500 bp in size have been deposited in GenBank GSS under Accession Numbers **HN108288-HN118367**. Approximately 70% matched with the Cot DNA Dataset 2. Similarity sequence analysis between BES and the *BmiGI* Version 2.0 reveals that a total of 502 BES (4.7%) aligned, at a length greater than 100 nucleotides and at greater than 90% identity (percent identity, PID), to 224 *BmiGI* entries or approximately 1.6% of the *BmiGI*.

The 10,582 BES were compared with the *I. scapularis* genome sequence, the nearest taxonomic whole genome sequencing project, comprising 369,492 scaffolds (supercontigs). Only 58 BES aligned with greater than 80% BES coverage and 80% PID. The number of BES found in comparative searches at specified thresholds were: 2,418 (23%) at an expected alignment value (e-value) of 1e-05 to the NCBI protein non-redundant (nr) database, 416 (4%) at an e-value 1e-20 to *I. scapularis* proteins (Lawson et al., 2009; Megy et al., 2009), 2,559 (24%) BES at an e-value 1e-20 to *R. microplus* gene indices (Guerrero et al., 2005), and 134 (1.2%) to the *I. scapularis* gene index (Ribeiro et al., 2006) at an e-value of 1e-20. The protein functional analyses by Gene Ontology (GO) classification are presented in Supplementary Table S2 and Supplementary Fig. S2.
For Dataset 4, 15 BAC clones were selected and sequenced to completion; 13 were based on hybridization of BAC library filter arrays to probes from known transcripts of interest involved in tick feeding and acaricide resistance and two were randomly selected BACs. These 15 BACs were subjected to sequencing and de-novo assembly. The sequencing of five of the BACs has been reported (Guerrero et al., 2010), and the sequences from the remaining 10 BACs have been deposited in GenBank (Accession Numbers HM748958-HM748967). Gene prediction analysis found 180 predicted genes with 919 predicted exons with an average exon size of 311 bp (Supplementary Fig. S2). From these predicted genes, there are 166 full-length genes comprised of 145 multiple exon genes containing both the initial and terminal predicted exons and 21 are single exon genes. Genes of particular interest to our research group that were identified in these BACs include cytochrome P450 (Guerrero et al., 2010), the permethrin-degrading carboxylesterase CzEst9 (Guerrero et al., 2010), papilin (Moolhuijzen et al., 2011), transmembrane protein 215-like, transpanin and serpin (Moolhuijzen et al., 2011). The Cot DNA dataset was also mapped to the BACs. Table 2 provides a summary of all 15 BACs, including the length of each assembled BAC, the number of Cot-selected DNA reads from Dataset 1 that mapped to each BAC using the next generation alignment tool BWA (Li and Durbin, 2010) with an alignment score > 10, the percentage of coverage of BAC sequence provided by the Cot reads, and the number of predicted genes and exons. As shown in Table 2, the percentage of Cot DNA coverage over these BACs ranged from 6% (BM-012-E08) to as high as 82% (BM-005-B21). Thirteen BACs had an average GC content between 44-48% (Table 2). One BAC (BM-012-E08) had a GC content of 58% and another BAC (BM-074-Random-F12) had a GC content of 40%. BAC BM-012-E08 contains rDNA and intergenic spacer and a significant proportion of highly
repetitive DNA. Present in the 15 BACs are the Ruka SINE elements identified previously in *Rhipicephalus appendiculatus* (Sunter et al., 2008) and a range of other interspersed repeats such as LINEs L2 and R1, LTR Gypsy and DNA transposons as found by RepeatMasker (Smit, A.F.A., Hubley, R., Green, P., 2004. RepeatMasker Open-3.0. 1996-2010 <http://www.repeatmasker.org> ). BAC BM-005-G14 contained a number of Ruka elements, which are a novel SINE element that was reported to occur frequently in both genomic and transcribed ixodid ticks (Sunter et al., 2008). A single 195 bp length Ruka element found in BM-004-G14 had 91% sequence identity to the 138 bp *R. appendiculatus* (Rap) Ruka (Genbank: EU018139.1 complement (9,947-10,084 bp)). A total of 70 Ruka (> 100 bp) elements were found in the RmiTr Version 1.0 transcriptome (Table 1 Dataset 8), while 409 were found in the assembled Cot DNA.

The remaining datasets are expression related. Dataset 5 consists of the *R. microplus* Gene Index BmiGI Version 2.1 (Quackenbush et al., 2001) entries that were extended by comparative analysis with the assembled Cot-selected genomic DNA (Guerrero et al., 2010). Datasets 6 and 7 consist of transcriptome sequence from adult female tick gut and ‘frustrated’ larvae libraries, respectively. These datasets were de novo assembled using Illumina short reads. We wanted to compare the transcriptome data contained in our datasets 6 and 7 with the BmiGI Version 2.1 to determine whether our data presented new transcripts that could be added to the current BmiGI to create a more comprehensive resource. Fig. 1 provides an overview of the CAP3 sequence clustering between datasets 6 and 7 and BmiGI Version 2.1 at greater than 98% identity and default settings. The Venn diagram shows that datasets 6 and 7 (Aus Rmi as noted in Fig. 1) contain 9,652 contigs not found in the BmiGI version 2.1. Dataset 8 is an updated *R. microplus* transcript data set made up of the
combination of the original BmiGI version 2.1 and Datasets 6 and 7, plus the
Subtraction Library data (GenBank: GO253189.1- GO253184.1, GE650059.1-
GE650181.1) and is referred to as RmiTr version 1.0, containing 28,893 sequences.
Finally, Dataset 9 consists of data from microarray experiments using Nimblegen
arrays (Rodriguez-Valle et al., 2010; Saldivar et al., 2008) to characterize the R.
microplus transcriptome responses to attaching and feeding upon Bos indicus and Bos
taurus cattle (Rodriguez-Valle et al., 2010).

3.2. CattleTickBase web resource

The CattleTickBase web resource provides a central point for the cattle tick
research community to access genome and transcriptome information on R.
microplus. The home page is shown in Fig. 2. This website contains a summary of the
datasets that have been generated to date and are available for download. It also
contains links to precomputed results, which are either figures from supplementary
material from publications or the datasets precomputed into the GBrowse genome
browser (Stein et al., 2002). To date, precomputed results include a summary of the
protein hits from similarity searches contained within the sequenced BACs (Dataset
4) as well as genes that were extended by the Cot DNA contigs that can be viewed
within GBrowse (Dataset 5).

A unique feature of our genome project web resource is the ability for the
research community to conduct their own sophisticated bioinformatics analysis
online. The open source YABI system (http://ccg.murdoch.edu.au/yabi) consists of a
front-end web application responsible for the user interface. Users create a secure
account and are free to access the datasets and analysis tools available within the
system. Users can create customized bioinformatics pipelines from the available tools and scripts that capture provenance information of the tools used, such as parameters used for each tool, and outputs of tools generated at each step. Fig. 3 shows the layout of the Design tab within YABI for constructing analysis workflows. The tools available are presented in the menu on the left. In the centre, dragging and dropping tools from the menu onto the workflow panel will construct a workflow. The example in Fig. 3 shows a screen shot of the creation of an automated workflow to search for G-Protein Coupled Receptors (GPCRs) in frustrated larvae transcripts that make up Dataset 7 (Table 1). GPCRs are an interesting family of membrane proteins that perform a range of critical biological functions in eukaryotes. Approximately 40% of the prescription pharmaceuticals target GPCRs (Filmore, 2004) and they are attractive as potential targets for acaricidal product development. Predicted open reading frames (ORFs) from the 6,082 assembled contig transcriptome from Dataset 7 were determined and the resulting sequences were searched using GPCRHMM (Wistrand et al., 2006). From a total of 17,230 predicted ORFs (≥ 200 bp), six ORFs were predicted to encode GPCRs (data not shown). A screen cast of this workflow can be found at (http://ceg.murdoch.edu.au/yabi). In CattleTickBase, the web-based analysis workflow environment (YABI) enables researchers to create sophisticated bioinformatics analysis pipelines from a diverse range of tools. For example, tools are available for multiple sequence alignment and assembly (including data from next-generation sequencing technologies), sequence searches, predictions for genes, rRNAs, tRNAs and small RNAs, repeat masking and various annotations. Also, the calculation of the Codon Adaptation Index for a given nucleotide sequence, given a reference codon usage table, can be used for predicting the level of expression of a
given gene and for making comparisons among codon usage in different organisms. *R. microplus* codon usage tables will be available for use in YABI.

4. Discussion

This paper provides an overview of the status of our collaborative efforts towards sequencing the *R. microplus* genome. Currently there is a scarcity of tick genome sequence and that limits progress in projects designed to address the significant worldwide impacts that *R. microplus* presents to cattle producers, both large and small. With the difficulties associated with sequencing the large and complex genome of *R. microplus*, an international collaboration has been formed to commence a coordinated effort of targeted sequencing of both genome and transcriptome with the longer-term view to obtain resources to sequence the complete genome of *R. microplus*.

As described in the results section, nine datasets have been generated and these contain the cumulative data our consortium has obtained which is relevant to *R. microplus* genome and transcriptome sequences. In broad terms, these are the Cot-filtration genomic DNA sequences, the BAC-associated sequences and the transcriptome data. The Cot-filtration process filters out highly repetitive DNA and allows the focus of resources on regions of the genome that are enriched for gene coding sequences. The result from our mapping of the Cot-selected DNA to each of the BACs is consistent with the Cot DNA experiments filtering out the highly repetitive DNA fraction. Our analysis demonstrated that Cot-filtration is an effective strategy to focus on gene-rich regions, because our Cot-filtered assembled contigs found matches with 50% of the *BmiGI* Version 2.1 Gene Index despite the assembled
Cot sequences (Table 1 Dataset 2) only representing 2% of the *R. microplus* genome.

The availability of contigs from the Cot genomic DNA data set to align to corresponding transcript sequences in BmiGI will enable the determination of intronic and promoter regions associated with the exonic regions contained in BmiGI.

The acquisition of BES data from *R. microplus* represents the first project of its type for this species. The 502 genes that were matched to the BES data represent a 16-fold increase over the expected number of matches based on the genome coverage of the BES. The BES represents 0.1% of the genome, yet matches to 1.6% of the BmiGI version 2.1 sequences. Further, the repetitive nature of the *R. microplus* genome has been determined by reassociation kinetics analysis and was shown to consist of 0.8% foldback, 30% unique DNA, 38% moderately repetitive DNA and 31% highly repetitive DNA (Ullmann et al., 2005). As the Cot DNA was selected to remove the highly repetitive sequence fraction (32% of the genome), this is consistent with 70% of BES alignment to the assembled Cot DNA contigs. This implies that the BES is high in gene content and accounted for a high percentage alignment to the gene-enriched Cot-selected DNA contigs. One possible reason for this is that the BAC libraries were made with *Mbo*I partially digested genomic DNA. *Mbo*I has GATC as the recognition site, and as GC-rich areas tend to be in gene-rich areas of genomes, *Mbo*I digestion which forms the BES may lend bias to these gene-rich areas.

The 15 BACs provide an insight into the genomic structure and organisation of *R. microplus*. The 15 BAC sequences are composed of 4.9% low complexity sequence and known retrotransposable elements, for example GYPSY and LTR. There is an average of 12 genes per BAC and this was a higher than expected ratio, given the estimated gene space. It is possible that these BACs, 13 of which were selected to be sequenced based on hybridization to known genes, occur in regions of
gene clusters. One particular BAC (BM-012-E08) had 6% of the BAC sequence length mapped by Cot DNA and the remaining unmapped BAC sequence contained highly repetitive intergenic regions nested between rDNA genes. In these selected 15 BACs a total of 97 genes out of 180 had significant alignments to known proteins. These included 28 hypothetical proteins of which 20 were found in *Ixodes scapularis*, five alignments to cytochrome P450 (*I. scapularis*), a receptor for egg jelly protein, a papilin (*Pediculus humanus corporis*), a zinc finger, five unknown *Tribolium* proteins, a serpin, an esterase and a transpanin. The remainder were comprised of polyproteins, helicases and transcriptases (transposable element-like), (gene predictions and annotation can be found in Supplementary Table S1 and Supplementary Fig. S1).

The content of the newly assembled *R. microplus* transcriptome for the cattle tick (Table 1 Dataset 8: *RmiTr* Version 1.0) contains 2,379 full-length sequences in common with the previous *R. microplus* gene index and an additional 9,652 novel full-length transcript sequences (Fig. 1). The *BmiGI* version 2.1 dataset consists of 9,851 contigs and 4,735 singletons. In our experience working with *BmiGI*, approximately one-third to one-half of the contigs encode full-length proteins while a very low percentage of the singletons encode full-length proteins. Thus, Dataset 8 should contain approximately 14,000 full-length transcripts. Further, the newly assembled transcripts for the cattle tick contained 3,829 sequences with orthologues to *I. scapularis* transcript sequences version 3.0 while there were 4,948 sequences in *I. scapularis* Gene Index Version 3.0 that had orthologues to sequences in *RmiTR* Version 1.0 (Table 3). As an example of the utility of CattleTickBase and *RmiTr* Version 1.0, we searched both *RmiTr* Version 1.0 and *I. scapularis* Gene Index Version 3.0 for sequences with similarity to esterases, cytochrome P450, and
glutathione S-transferases, metabolic proteins that often play roles in tick resistance to acaricides. The results are shown in Table 3 and Supplementary Data S1-S3.

CattleTickBase is designed to become the comprehensive bioinformatics web resource for *R. microplus*. CattleTickBase currently contains over 1.8 Gbp of genomic sequence from *R. microplus*. Most of this sequence was derived from the Cot-selected genomic DNA. This DNA was selected to yield the unique gene-enriched fraction of the genomic DNA from *R. microplus* (Guerrero et al., 2010). Thus, if we assume the 30% unique fraction value from Ullmann et al. (2005), and also assume that our 1.8 Gbp of Cot-selected data primarily sources from the unique fraction (Table 1 Dataset 1), then with the *R. microplus* genome size of 7.1 Gbp, 30% of unique fraction would represent 2.1 Gbp. Thus continuing the assumptions, CattleTickBase can be considered to represent 0.86X coverage of the unique fraction (gene-enriched fraction) of the *R. microplus* genome. However our Cot filtration dataset, although effective in the recovery of cattle tick genomic DNA regions enriched in gene coding sequences, has a drawback in that the low coverage has resulted in a somewhat fragmented sequence assembly. This needs to be rectified by deeper sequencing of the Cot-selected DNA or whole genome sequencing. Additionally, we expect genetic variations amongst *R. microplus* populations from different countries or ecological regions and this is necessarily an important consideration when defining consensus reference data sets for *R. microplus*. Most of the data in CattleTickBase is derived from North American *R. microplus*, although datasets 6 and 7 in Table 1 are from Australian ticks. An example of a well-studied *R. microplus* transcript with sequence that varies among different population is *Bm86*. Freeman et al. (2010) found 8.3% sequence variation between *Bm86* isolated from *R. microplus* ticks from South Texas,
USA and from the Australian Yeerongpilly strain. Andreotti et al. (2008) reported similar geographical differences in Bm86 isolated from South American populations. Nevertheless, we believe CattleTickBase is a significant resource for the tick research community that should facilitate tick research in a number of areas. Not only does CattleTickBase provide standard features of a genome sequencing project browser and a BLAST interface; it also contains an online web-based analysis environment (YABI) that enables the scientific community to conduct sophisticated bioinformatics analysis. YABI’s ease of use and facility for results to be easily downloaded and used in other bioinformatics analysis environments makes it a flexible web-based bioinformatics environment. To our knowledge, this type of environment is not offered elsewhere. With the advent of third generation sequencing technologies that promise longer reads than current 454 and Illumina technologies, we expect to focus upon whole genome sequencing and targeted transcriptomic analysis to further advance the R. microplus genome sequencing project. Naturally, progress will be dependent on obtaining the necessary resources (both financial and scientific) to further sequence these more difficult regions of the cattle tick genome. As obtained, this information will be integrated into CattleTickBase to provide the scientific community with access to the latest information from the R. microplus genome-sequencing project.

In summary, we have acquired and assembled genomic DNA and transcriptomic sequence data into contigs which represent over 1.8 Gbp of DNA from gene-enriched regions of the R. microplus genome. The data was compiled into a resource called CattleTickBase and a web-based YABI resource was developed to enable the scientific community to access our databases. The YABI tool will facilitate
access and manipulation of cattle tick genome sequence data and, as the genome of *R. microplus* proceeds to completion, the CattleTickBase resource will be updated.

**Acknowledgements**

The authors acknowledge funding support from the Beef CRC and the National and International Research Alliances Program (DEEDI, Queensland Government Smart Futures Funds, Australia) and U.S. Department of Agriculture (USDA)-ARS Knipling Bushland US Livestock Insect Research Laboratory CRIS Project Number 6205-32000-031-00 for the tick BAC and BES. We further acknowledge technical assistance from Ms. Kylie G. Bendele, USDA-ARS Kerrville, TX, USA and Dr. Zenaida Magbanua, Mississippi State University, USA and Dr. Louise Jackson from Biosecurity, DEEDI, Australia for providing Australian ticks. Thanks to Dr. Rudi Appels for helpful discussions during data analysis and manuscript preparation. USDA is an equal opportunity employer.
References


Bm86 midgut protein sequence variation in South Texas cattle fever ticks.


Legends to Figures

Fig. 1. Venn diagram summarizing the overlap between tick transcripts (S1 and S2) and the Rhipicephalus microplus (Rmi) Gene Index (BmiGI Version 2.1). Transcript sequences were clustered between Australian datasets 6 and 7 (Table 1) and the USA BmiGI Version 2.1 gene index (http://compbio.dfci.harvard.edu/cgi-bin/tgi/gimain.pl?gudb=b_microplus) at greater than 98 percent identity (PID). The Venn diagram shows that the Australian datasets 6 and 7 contain 9,652 transcripts not found in the BmiGI.

Fig. 2. Screen shot of the CattleTickBase home page. The CattleTickBase main web page is a major resource for Rhipicephalus microplus data and analysis. On the CattleTickBase home page pre-computed analyses can be viewed under the "View Analyses" box; this includes genome browsers and full transcript annotations. Rhipicephalus microplus sequence datasets are freely available for download from the "Download Data" box. In the "Analysis Tools" box R. microplus datasets are available for researchers to conduct their own analysis through a number of listed bioinformatics applications and create customized bioinformatics pipelines. Users are free to access the datasets and analysis tools available within the system, or can create a secure account.

Fig. 3. YABI workflow analysis example for the identification of G protein-coupled receptors (GPCRs) from transcript sequences in Dataset 7 of Table 1. The flow diagram on the left hand side shows a simplified three-step bioinformatics workflow, Step 1. Select a file of nucleotide sequences, Step 2. Translate all available open reading frames (ORF) to amino acid sequences, Step 3. Search for GPCRs in the translated ORF. On the right hand side of the figure is a screen capture of the YABI
online application; in the "Jobs" tab the automated three-step analysis pipeline labelled "GPCR Rmi FL" is running. Steps 1 and 2 have completed successfully (green circle with white mark) and step 3 is running, waiting for completion indicated by the green bar. The remaining tabs include a "design" tab to create workflows and a "files" tab where all files and results are stored.
Table 1. Cattle Tick genome sequence and other relevant datasets available on CattleTickBase.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Description</th>
<th>No. of sequences</th>
<th>No. of bp</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cot-selected genomic DNA seqs.</td>
<td>7,289,230</td>
<td>1,798,400,445</td>
<td>Guerrero et al., 2010</td>
</tr>
<tr>
<td>2</td>
<td>Cot-selected genomic DNA assembly</td>
<td>175,226</td>
<td>144,709,321</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>BAC(^a) end sequences</td>
<td>10,582</td>
<td>7,290,530</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Full-length sequenced BACs (15 BAC clones)</td>
<td>15</td>
<td>1,502,117</td>
<td>Guerrero et al., 2010, Moolhuijzen et al., 2011</td>
</tr>
<tr>
<td>5</td>
<td>BmiGi(^b) contigs extended by Cot-selected seqs.</td>
<td>3,913</td>
<td>4,240,351</td>
<td>Guerrero et al., 2010</td>
</tr>
<tr>
<td>6</td>
<td>Tick gut transcriptome</td>
<td>11,333</td>
<td>9,228,737</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Frustrated larval transcriptome</td>
<td>6,082</td>
<td>3,617,080</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Assembled transcriptome \textit{RmiTr}^c Version 1.0</td>
<td>28,893</td>
<td>24,673,517</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>NimbleGen microarray analyses GEO dataset GSE20605 (10 arrays)</td>
<td></td>
<td></td>
<td>Rodriguez-Valle et al., 2010</td>
</tr>
</tbody>
</table>
a Bacterial Artificial Chromosome

b Boophilus microplus Gene Index at http://compbio.dfci.harvard.edu/cgi-bin/tgi/gimain.pl?gudb=b_microplus

c Rhipicephalus microplus transcriptome
Table 2. Statistics of the 15 sequenced Bacterial Artificial Chromosomes (BACs) compared with Cot-selected genomic DNA.

<table>
<thead>
<tr>
<th>BAC</th>
<th>BAC length (bp)</th>
<th># Mapped Cot DNA reads</th>
<th>BAC Cot % coverage</th>
<th>No. of Genes/No. of Exons</th>
<th>GC content %</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM-005-B21</td>
<td>92,305</td>
<td>4,068</td>
<td>82</td>
<td>12/42</td>
<td>45</td>
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<tr>
<td>BM-005-G14</td>
<td>135,319</td>
<td>3,138</td>
<td>58</td>
<td>5/76</td>
<td>44</td>
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<tr>
<td>BM-013-M17</td>
<td>90,249</td>
<td>2,286</td>
<td>64</td>
<td>9/44</td>
<td>46</td>
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<tr>
<td>BM-026-P08</td>
<td>108,580</td>
<td>2,649</td>
<td>70</td>
<td>11/62</td>
<td>45</td>
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<tr>
<td>BM-031-L02</td>
<td>125,915</td>
<td>3,115</td>
<td>65</td>
<td>14/64</td>
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<tr>
<td>BM-118-H10</td>
<td>92,057</td>
<td>3,658</td>
<td>72</td>
<td>10/50</td>
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<td>BM-004-A11</td>
<td>103,837</td>
<td>5,085</td>
<td>79</td>
<td>15/66</td>
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<tr>
<td>BM-006-B07</td>
<td>102,433</td>
<td>4,416</td>
<td>79</td>
<td>12/53</td>
<td>46</td>
</tr>
<tr>
<td>BM-010-J12</td>
<td>172,065</td>
<td>3,014</td>
<td>53</td>
<td>24/124</td>
<td>48</td>
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<tr>
<td>BM-012-E08</td>
<td>51,489</td>
<td>146</td>
<td>6</td>
<td>3/14</td>
<td>58</td>
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<tr>
<td>BM-074-Random-F12</td>
<td>95,687</td>
<td>1,534</td>
<td>61</td>
<td>15/40</td>
<td>40</td>
</tr>
<tr>
<td>BM-077-Random-J09</td>
<td>103,645</td>
<td>2,777</td>
<td>59</td>
<td>12/52</td>
<td>47</td>
</tr>
<tr>
<td>BM-129-CzEst9-N14</td>
<td>126,498</td>
<td>3,919</td>
<td>64</td>
<td>14/71</td>
<td>46</td>
</tr>
<tr>
<td>BM-066-M07</td>
<td>151,523</td>
<td>3,608</td>
<td>51</td>
<td>16/70</td>
<td>46</td>
</tr>
<tr>
<td>BM-077-G20</td>
<td>94,838</td>
<td>1,692</td>
<td>54</td>
<td>8/64</td>
<td>46</td>
</tr>
</tbody>
</table>

*a* The number of Cot DNA reads mapped onto BAC sequence

*b* The percentage coverage of BAC sequence length by aligned Cot DNA reads

*c* Count of predicted genes and total number of exons for each BAC sequence
Table 3. *Ixodes scapularis (Isc)* transcript orthologous sequences in the *Rhipicephalus microplus (Rmi)Tr* Version 1.0.

<table>
<thead>
<tr>
<th>Data</th>
<th><em>RmiTr</em>1.0 Total Seqs.</th>
<th>Orthologues with <em>Isc</em></th>
<th><em>Isc</em> Gene Index Version 3.0 Total Seqs.</th>
<th>Orthologues with <em>RmiTr</em></th>
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</thead>
<tbody>
<tr>
<td>Overall Statistics</td>
<td>28,893</td>
<td>3,829</td>
<td>38,392</td>
<td>4,948</td>
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<tr>
<td>Cytochrome P450</td>
<td>115</td>
<td>55</td>
<td>357</td>
<td>235</td>
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<tr>
<td>Esterases</td>
<td>81</td>
<td>14</td>
<td>133</td>
<td>33</td>
</tr>
<tr>
<td>Glutathione S-transferases</td>
<td>39</td>
<td>28</td>
<td>96</td>
<td>60</td>
</tr>
</tbody>
</table>
Figure 1
Introduction to CCG Rhipicephalus microplus Bioinformatics

A collaborative project between the State of Queensland (Department of Employment Economic Development and Innovation), Murdoch University's Centre for Comparative Genomics, and the United States Department of Agriculture Agricultural Research Service has generated a large amount of novel data and led to the creation of this online R. microplus genome resource. Rhipicephalus microplus. The cattle tick (formerly known as Boophilus microplus) is the most significant ectoparasite of cattle worldwide causing production losses and hide damage. Ticks are also vectors for highly pathogenic organisms including protozoan parasites Babesia bovis and B. bigemina causing bovine babesiosis and rickettsia Anaplasma marginale causing bovine anaplasmosis.

Research Aims
Integration of novel vector sequences and analyses to identify the genomic mechanisms to effectively control vector R. microplus livestock infections.

Project site contains
This site contains access to data, analysis and to tools specific to the analysis of Rhipicephalus microplus. Data types include EST, BAC, and Genomic DNA sequences, microarray data and mRNA studies. An interactive Data Entry and Analysis window (YABE). Get started using our online tutorial (link) and online help. Or go direct to View Analysis tools.
Figure 3 colour

Select a sequence file

Translate nucleotide to protein

Search for G-Protein Couple Receptors
Select a sequence file

Translate nucleotide to protein

Search for G-Protein Couple Receptors
Consortium formed to begin sequencing cattle tick genome. Acquired and assembled over 1.8 Gbp from gene-enriched regions of genome. Web resource developed for access to data through web-based environment.