Whole transcriptome analysis of the poultry red mite *Dermanyssus gallinae* (De Geer, 1778)

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(*Received 23 April 2013; revised 12 June and 22 July 2013; accepted 22 July 2013; first published online 18 October 2013*)

SUMMARY

Although the poultry red mite *Dermanyssus gallinae* (De Geer, 1778) is the major parasitic pest in poultry farming causing substantial economic losses every year, nucleotide data are rare in the public databases. Therefore, *de novo* sequencing covering the transcriptome of *D. gallinae* was carried out resulting in a dataset of 232 097 singletons and 42 130 contiguous sequences (contigs) which were subsequently clustered into 24 140 isogroups consisting of 35 788 isotigs. After removal of sequences possibly originating from bacteria or the chicken host, 267 464 sequences (231 657 singletons, 56 contigs and 35 751 isotigs) remained, of which 10·3% showed homology to proteins derived from other organisms. The most significant Blast top-hit species was the mite *Metaseiulus occidentalis* followed by the tick *Ixodes scapularis*. To gain functional knowledge of *D. gallinae* transcripts, sequences were mapped to Gene Ontology terms, Kyoto Encyclopedia of Gene and Genomes (KEGG) pathways and parsed to InterProScan. The transcriptome dataset provides new insights in general mite genetics and lays a foundation for future studies on stage-specific transcriptomics as well as genomic, proteomic, and metabolomic explorations and might provide new perspectives to control this parasitic mite by identifying possible drug targets or vaccine candidates. It is also worth noting that in different tested species of the class Arachnida no 28S rRNA was detectable in the rRNA profile, indicating that 28S rRNA might consists of two separate, hydrogen-bonded fragments, whose (heat-induced) disruption may led to co-migration with 18S rRNA.

Key words: Next generation sequencing, 454 pyrosequencing, *de novo* assembly, transcriptome analysis, parasitic mite, Acari, Dermanyssidae, 28S rRNA.

INTRODUCTION

Dermanyssus gallinae (De Geer, 1778), the poultry red mite, is a haematophagous, transient ectoparasite infesting domestic and wild birds worldwide. In absence of their preferred hosts, they may also infest domestic animals like dogs, cats, rodents, horses as well as humans (Brockis, 1980). *Dermanyssus gallinae* parasitizes during the night and hides in crevices during the day. If no hosts are available, the mites can starve for months. Mite bites cause itching, dermatitis, anaemia, weight loss, decrease in egg production and they increase the incidence of aggressive pecking and cannibalism in poultry stock (Kirkwood, 1967; Chauve, 1998). As *D. gallinae* reproduces very rapidly, chicken are often parasitized by huge numbers of mites and can even die due to substantial blood losses. The estimated annual costs related to damage due to infestation with *D. gallinae* range from €3 million for the UK only and €130 million throughout Europe, whereas the annual economic losses in Japan sum up to €66·85 million (Mul *et al.* 2009; Sparagano *et al.* 2009). Under optimal conditions, the mite completes the life cycle within one week, resulting in the rapid establishment of dense populations (Wood, 1917). When additionally considering vector function, for example for *Erysipelothrix rhusiopathiae* and *Salmonella enteritidis*, and relevance as a zoonotic agent, *D. gallinae* appears as one of the most important parasites in intensive poultry management (Chirico *et al.* 2003; Valiente Moro *et al.* 2007). Even though more than 35 acaricide compounds are available against *D. gallinae* (Chauve, 1998), control of the parasite is extremely difficult due to various reasons: Affected farms are confronted with problems in controlling the parasite due to acaricide resistance (Zeman and Zelezny, 1985; Beugnet *et al.* 1997; Nordenfors *et al.* 2001; Marangi *et al.* 2009) as well as food safety regulations as acaricide residues may remain in animal products. As no acaricide is registered for use on poultry, mite control can only rely on treatment of facilities. Therefore, the development of alternative control methods, such as a recombinant vaccine, would be desirable. However, extrapolation of gained knowledge of other haematophagous parasites to *D. gallinae* resulted in moderate control success. In a study by Harrington *et al.* (2009*a*), where poultry was immunized with *Rhipicephalus microplus* (formerly *Boophilus microplus*) tick protein

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Parasitology (2014), **141**, 336–346. © Cambridge University Press 2013
doi:10.1017/S0031182013001467

Bm86, a *D. gallinae* mortality of 35·1% compared to the control group was found. Vaccination with subolesin originating from the mosquito *Aedes albopictus* resulted in 23% *D. gallinae* mortality only. In another immunization trial using soluble proteins derived from *D. gallinae*, 50·6% mite mortality was reached 17 h after feeding (Harrington *et al*. 2009*b*). These results indicate that further research is needed to discover new drug targets or vaccine candidates against *D. gallinae*. For this purpose, genetic information is required which is rare for the poultry red mite. Until now (April 2013), about 2100 partial nucleotide sequences derived from only 11 different genes are available in the GenBank sequence database of the National Center for Biotechnology Information (NCBI). To the best of our knowledge, only two transcriptome analyses of mite species were performed, covering the entire transcriptome by investigating different mite stages. Cabrera *et al*. (2010) performed 454 pyrosequencing of mobile stages of the predatory phytoseiid mite *Phytoseiulus persimilis* resulting in a transcriptome composed of 12 556 contiguous sequences. Hoy *et al*. (2012) also applied 454 pyrosequencing to *Metaseiulus occidentalis*, another predatory phytoseiid mite. The authors obtained a transcriptome of 255·6 Mbp in size composed of 74 172 non-redundant sequences including 30 691 contigs and 43 481 singletons. More common than whole transcriptome analyses are studies investigating mites' transcript profiles in terms of acaricide exposure (Liu *et al*. 2011; Niu *et al*. 2012).

For ticks, which are closely related to mites, mainly transcriptome studies of specific organs, like the synganglion and associated neurosecretory organs as well as salivary glands or midgut, are available (Alarcon-Chaidez *et al*. 2007; Anderson *et al*. 2008; Bissinger *et al*. 2011; Ribeiro *et al*. 2012). Even though *D. gallinae* shares anatomical similarities to ticks, for example the intestinal tract (Harrison and Foelix, 1999), considerable differences between ticks and mites are assumed (Cabrera *et al*. 2010). For example, the genome size of the deer tick, *Ixodes scapularis*, is close to 1·765 Gbp (www.vectorbase.org) in comparison with mite genomes ranging from 75 Mbp for the predatory mite *M. occidentalis* (Hoy, 2009), 90 Mbp for the herbivorous pest *Tetranychus urticae* and 294 Mbp (Grbić *et al*. 2011) for the parasitic mite *Varroa destructor* (Cornman *et al*. 2010). The genome size of *D. gallinae* is still unknown.

To partially close the gap of genetic knowledge of *D. gallinae*, *de novo* transcriptome sequencing using 454 pyrosequencing was performed. The dataset provides valuable information on mites' biology on the molecular level and might provide prospects for novel intervention methods against parasitic mites such as drug or vaccine development.

MATERIALS AND METHODS

Mite differentiation and isolation of total RNA

An acaricide-susceptible *D. gallinae* isolate maintained at the Institute for Parasitology of the University of Veterinary Medicine Hannover was used for 454 whole transcriptome pyrosequencing. Feeding of *D. gallinae* on hens for maintenance was permitted by the ethics commission of the Lower Saxony State Office for Consumer Protection and Food Safety under reference number AZ 33-42502-05-02A159. Twenty hens originating from a laying hen farm were housed in a stable with outdoor access (total area of about 30 m^2). The stable was interspersed with straw for pawing and fitted with perches for resting and nests for egg laying. Food and water were provided *ad libitum*. Once a week hens were placed in a box in which *D. gallinae* was kept. Mites were allowed to feed for 3 h in the dark. Subsequently, lights were turned on to assure that mites return to the boxes' niches and the hens were brought back into their stable after additional 3 h.

All stages and sexes of starved as well as freshly fed *D. gallinae* mites were included in RNA isolation. For stage and sex differentiation, the mites were immobilized in a Petri dish placed on ice. Differentiation of the mites' developmental stage and sex was carried out under a dissecting microscope according to the description by Pound and Oliver (1976). Individual mite stages were collected in glass bead tubes (Precellys Glas-Kit 0·5 mm, Peqlab Biotechnologie, Erlangen, Germany) placed on ice. In total 253 eggs, 200 larvae, 150 protonymphs, 150 deutonymphs, 100 adult males and 50 adult females were collected from a batch of starved mites. Additionally, one pooled sample of fresh fed mites containing all blood sucking stages was prepared for total RNA isolation. The majority of collected fed mites were immature stages (proto- and deutonymphs), followed by female adults and males. Weights of mite material included in RNA isolation are listed in Additional file 1 – in Online version only. Preparation of mites was carried out by adding 600 μL RLT-buffer (RNeasy® Mini Kit, Qiagen, Hilden, Germany) and 6 μL 2-mercaptoethanol (Roth, Karlsruhe, Germany) with a subsequent homogenization step via two cycles of 10 s at 5000 rpm using Precellys® 24 (Peqlab, Erlangen, Germany). Total RNA was isolated using the RNeasy® Mini Kit according to the manufacturer's instructions with an additional on-column DNase digestion step using the RNase-Free DNase set (Qiagen, Hilden, Germany).

Total RNA concentration and purity were determined using the NanoDrop ND-1000 spectral photometer (Peqlab, Erlangen, Germany). Furthermore, total RNA parameters and integrity were checked with the Agilent 2100 Bioanalyzer by the use of the Agilent RNA 6000 Nano Kit (Agilent

Technologies, Böblingen, Germany). Since no 28S rRNA band of *D. gallinae* total RNA could be visualized, total RNA of an arachnid (the cellar spider *Pholcus phalangioides*), an acarid (the ornate cow tick *Dermacentor reticulatus*) and mammalian organism (cattle) was run against pooled *D. gallinae* total RNA to check RNA integrity.

cDNA synthesis and normalization

For cDNA synthesis, total RNA of all developmental stages and sexes of starved mites as well as fed mites was pooled. Total RNA (1·5 μg) was used for cDNA synthesis with modified protocol of MINT cDNA synthesis kit (Evrogen, cat. No. SK001). The 1 μL of 3′primer was replaced with 1 μL of primer PolTdeg (5′-AAG CAG TGG TAT CAA CGC AGA GTA CTT TTG TTT TTT TTT CTT TTT TTT TTV N-3′). Synthesized cDNA was normalized with a modified protocol of TRIMMER cDNA Normalization kit (Evrogen, cat. no. NK001). The 2 μL of PCR Primer M1 provided in the kit were substituted for 1 μL of primer M1ACGG (5′- AAG CAG TGG TAT CAA CGC AGA GTA CGG – 3′) and 1 μL of primer polTM1 (5′-AAG CAG TGG TAT CAA CGC AGA GTA CTT TTG TCT TTT GTT CTG TTT CTT TTV N-3′).

454 sequencing

Sequencing libraries were prepared with GS Rapid Library Kit (Roche, cat. no. 05.608.228.001) and GS Rapid Library MID Adaptors Kit (Roche, 05 619 211 001) using 500 ng of normalized cDNA according to the manufacturer's protocol. The sequencing reactions were performed using a Roche 454 Genome Sequencer FLX with the GS Titanium Sequencing Kit XLR70 (Roche, cat. no. 05.233.526.001) using two big regions of the GS Titanium Pico Titer Plate Kit (70 × 75) (Roche, cat. no. 05.233.682.001), according to the manufacturer's instructions. Image and signal processing were done using GS FLX SW v 2.5.3, gsRunProcessor fullProcessing.

Sequence assembly

454 raw reads were first trimmed of adaptor sequences used in cDNA library preparation and normalization with 454 Sequencing System Software (GS *De Novo* Assembler v 2.5.3). A megablast search was performed to make sure that the trimmed reads were clean of these adaptor sequences. The trimmed reads were further cleaned using Seqclean (http://compbio.dfci.harvard.edu/tgi/software/) to remove sequences of low complexity and sequences containing more than 3% of uncalled bases. Tails of polyA/Ts were also trimmed at this step. Processed reads were assembled using the Newbler gsAssembler (v 2.5.3) with default parameter combinations. 454 raw reads have been submitted to NCBI Short Read Archive (SRA; experiment accession number SRX222259). Assembled reads were submitted to NCBI Transcriptome Shotgun Assembly (TSA) Sequence Database and has been deposited at DDBJ/EMBL/GenBank under the project accession GAIF00000000. The version described in this paper is the first version, GAIF01000000.

Identification of homologues and contamination

For identification of putative homologues, obtained singletons, contigs and isotigs from *D. gallinae* were compared with the NCBI nr-database using the BLASTx algorithm as implemented in the blastall program package (BLAST+) provided by NCBI. The E-value cut off was set to $1{\cdot}00 \times 10^{-6}$. Resulting BLASTx hits were analysed for their species to exclude nucleotide sequences potentially originating from contaminating species from the dataset ($\geqslant$ 85% similarity to avian or bacterial sequences). Singletons, contigs and isotigs were parsed to their BLASTx results using the MEtaGenome ANalyzer (MEGAN 4) software (Huson *et al*. 2007, 2011) by setting default parameters with exception of LCA parameters (Min support filter: 1; Min score filter: 100). Again, sequences representing possible contaminants were checked and excluded from the dataset.

Functional sequence annotation

The Blast2Go (b2 g) software suite (Conesa *et al*. 2005; Gotz *et al*. 2008) was used to predict functions of *D. gallinae* transcripts. When assigning Gene Ontology terms (Ashburner *et al*. 2000) sequence filters (seq filter) as well as subcategory levels were set to take the large number of *D. gallinae* sequences into account. Metabolic pathway mapping was done using the Kyoto Encyclopedia of Gene and Genomes (KEGG) database resource (Kanehisa *et al*. 2006, 2008).

To identify protein domains, the nucleotide sequences were conceptually translated via ESTScan (Lottaz *et al*. 2003) and subsequently analysed via InterProScan (Hunter *et al*. 2011). Parasitiformes mRNA and UniGene sequences were downloaded from NCBI to generate the customized scoring matrix used in ESTScan translation.

RESULTS AND DISCUSSION

Isolation of total RNA

Total RNA was isolated from all stages and sexes of starved as well as freshly fed mites. As shown in Additional file 1 – in Online version only, isolation resulted in amounts between 80 ng μL^{-1} and

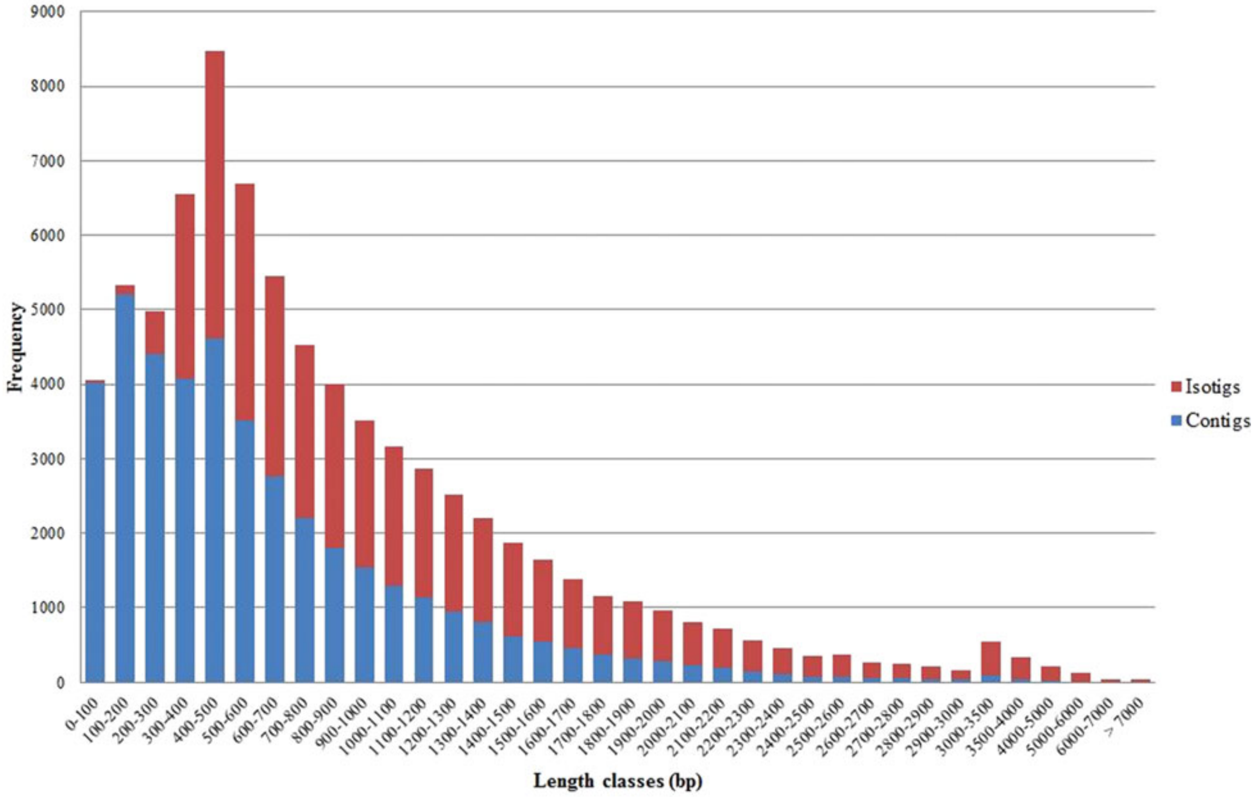

Fig. 1. Distribution of contig and isotig lengths of *D. gallinae* sequences according to different length classes.

373 ng μL^{-1} of total RNA from individual stages with 260/280 ratios as well as 260/230 ratios >2, indicating high RNA purity. Agilent 2100 Bioanalyzer results showed a distinct band corresponding to the 18S rRNA, whereas no 28S rRNA band appeared and the 28S rRNA/18S rRNA ratio was 0. Therefore, total RNA band pattern was compared with those of two other members of the class Arachnida, which were the ixodid ornate cow tick (*Dermacentor reticulatus*) and the cellar spider (*P. phalangioides*) as well as total RNA of a mammal (cattle). Again, the 28S rRNA band was missing in arachnid total RNA, whereas the mammalian one appeared as two distinct bands corresponding to the 18S and 28S rRNA. Additional file 2 – in Online version only visualizes the comparison of total RNA band pattern of starved and fed mites as well as bovine blood. A similar result was observed for *Sarcoptes scabiei* mites by Kuhn (2005), who obtained a 28S rRNA/18S rRNA ratio of 0·07 by use of the Agilent 2100 Bioanalyzer but no visible 28S rRNA band on formaldehyde agarose gels. It is known for insects that upon heating 28S rRNA hydrogen bonds are disrupted (summarized by Winnebeck *et al*. 2010). The two resulting similar sized fragments migrate closely with 18S rRNA resulting in a single rRNA peak instead of two clear peaks on an Agilent 2100 Bioanalyzer chip, when – as recommended by the manufacturer – RNA samples were heat-denatured prior to chip loading. When insect samples were loaded without heat-denaturation, the expected 18S and 28S rRNA peaks were observed (Winnebeck *et al*. 2010). However, when comparing Agilent 2100 Bioanalyzer rRNA profiles of heat-denatured to non-denatured *D. gallinae* RNA, the same electrophoretic profile was observed (data not shown). This observation may suggest that denaturation of *D. gallinae* 28S rRNA happened prior to total RNA isolation, e.g. during homogenization. Even though glass bead homogenization was kept comparatively short and at low speed (2 × 10 s at 5000 rpm; available Precellys$^{®}$ application database recommendation for tick RNA: 3 × 30 s at 6000 rpm), glass bead grinding may have been associated with denaturing heat generation. Another explanation could be that in mites and other arachnid taxa 28S rRNA transcription is low to start with.

454 pyrosequencing and de novo assembly

A normalized cDNA library was constructed from pooled total RNA including all stages, sexes of starved as well as freshly fed mites, and was subsequently used for two 454 pyrosequencing runs. Sequences passing basic quality standards of both runs were combined to a dataset containing ~446 Mbp of sequences comprising 1 518 941 reads with a mean length of 295 bp. Of these processed reads, 81·47% were aligned and 75·13% were assembled. *De novo* assembly and clustering of the reads resulted in 232 097 singletons (average size of 285 bp) and 42 130 contiguous sequences (contigs;

Table 1. Summary and statistics of *D. gallinae* 454 pyrosequencing and *de novo* assembly

Run metrics	Total raw reads	1 532 379
	Total bases	480 188 551
	Reads after quality control and trimming	1 518 941
	Bases entering assembly	447 581 354
Assembly	Aligned reads	1 237 427 (81·47%)[a]
	Aligned bases	363 645 048 (81·25%)[b]
	Assembled reads	1 141 153 (75·13%)[a]
	Fully assembled	1 045 365 (68·82%)[a]
	Partially assembled	95 788 (6·31%)[a]
	Singleton	232 097 (15·28%)[a]
	Repeat	611 (0·04%)[a]
	Outlier	30 011 (1·98%)[a]
	Too short	19 281 (1·27%)[a]
Isogroup metrics	Number of isogroups	24 140
	Average contig content of isogroups	1·70
	Largest contig content of isogroups	41
	Number of isogroups with one contig	18 506
	Average isotig content of isogroups	1·5
	Largest isotig content of isogroups	96
	Number of isogroups with one isotig	18 714
Isotig metrics	Number of isotigs	35 788
	Average contig content of isotigs	2·2
	Largest contig content of isotigs	14
	Number isotigs with one contig	19 509
	Number of bases	40 786 369
	Average isotig size	1139 bp
	N50 isotig size	1444 bp
	Largest isotig size	13 249 bp
Large contig metrics (contig size ⩾ 500 bp)	Number of contigs	19 813
	Number of bases	20 619 359
	Average contig size	1040 bp
	N50 contig size	1144 bp
	Largest contig size	7446 bp
All contig metrics	Number of contigs	42 130
	Number of bases	26 143 288
	Average contig size	621 bp

[a] Percentage of 'Reads after quality control and trimming'.
[b] Percentage of 'Bases entering assembly'.

average size of 621 bp) of which 19 813 represented large contigs (longer than 500 bp, average size of 1040 bp). Clustering of the 42 130 contigs revealed 24 140 isogroups consisting of 35 788 isotigs (average size of 1139 bp) which were assumed to represent gene transcripts. Frequencies of contig and isotig lengths obtained from *D. gallinae* sequences are shown in Fig. 1. Detailed overview about 454 pyrosequencing and *de novo* assembly statistics is given in Table 1. Analyses of mite transcriptomes are rarely found. The first 454 pyrosequencing-based whole transcriptome analysis of a mite species was performed by Cabrera *et al.* (2010), who obtained 12 556 contigs (average size of 935 bp) for the phytoseiid predatory mite *P. persimilis*. In another study by Hoy *et al.* (2012), 30 691 contigs (average size of 681 bp) were obtained by 454 pyrosequencing for the *M. occidentalis*, which is a phytoseiid predatory mite as well. Compared with 42 130 contigs derived from *D. gallinae* a smaller contig number was found for the phytoseiid predatory mite species with *P. persimilis* showing the smallest contig number. Contig number differences may be due to the fact that Cabrera *et al.* (2010) analysed mobile stages of *P. persimilis* mites only, whereas all developmental stages including eggs were included in the case of *D. gallinae* and *M. occidentalis*. Another reason might be that the three mite species are within the suborder Dermanyssiae, but represent different families [Dermanyssidae (*D. gallinae*) *vs* Phytoseiidae (*P. persimilis* and *M. occidentalis*)] which may explain differences in transcriptome size. However, contig number differences may also result from technical reasons: While *P. persimilis* and *M. occidentalis* data are based on a single 454 pyrosequencing run, two runs were performed for *D. gallinae*.

Removal of potential contaminating sequences from the dataset

To ensure that only mite transcripts were included in subsequent functional annotations, sequences

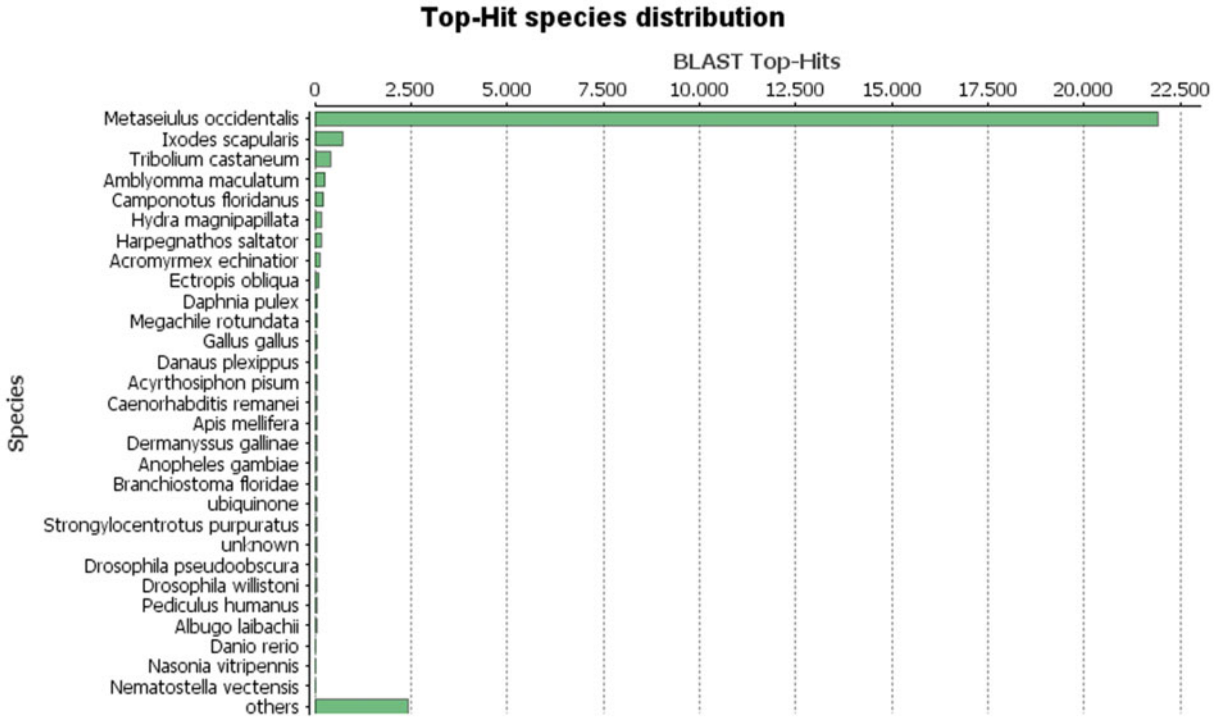

Fig. 2. BLASTx top-hit species distribution of the *D. gallinae* transcriptome using an *E*-value cut off of $1{\cdot}00 \times 10^{-6}$.

potentially originating from contaminants like the chicken host or microbial endosymbionts were identified based on BLASTx results and MEGAN (MEtaGenome Analyzer) analysis. MEGAN assigned 108 sequences to avian taxa and 374 to bacteria (assignments of isotig and singleton species level taxonomy are shown in Additional files 3 and 4 – in Online version only, respectively). Analysis of BLAST results revealed 570 and 883 sequences showing similarities with avian and bacterial sequences, respectively. Application of the similarity cut off value of ⩾85% resulted in subtraction of 246 bacteria homologues (0·09%) and 231 *Gallus gallus* homologues (0·09%). A number of 64 *G. gallus* homologues (0·2%) showed less than 85% similarity and thus remained in the dataset, which finally consisted of a total of 267 464 sequences (231 657 singletons, 56 contigs and 35 751 isotigs) for further analyses.

Functional annotation

From the 267 464 *D. gallinae* sequences available, a number of 27 529 (10·3%) showed significant BLASTx matches (*E*-value $< 1{\cdot}00 \times 10^{-6}$) with protein sequences deposited in the non-redundant GenBank database. In more detail, 15 069 of 231 657 (6·5%) singletons, 25 of 56 (44·6%) contigs and 15 069 of 35 751 (42·2%) isotigs showed similarities to published protein sequences. The remaining 89·7% *D. gallinae* sequences were assigned as 'novel'. By contrast, Cabrera *et al.* (2010) and Hoy *et al.* (2012) obtained higher numbers of BLASTx matches for *P. persimilis* (4862/12 556 sequences, 38·7%) and *M. occidentalis* mites (25 888/74 172 sequences, 34·9%), which might result from their smaller contig numbers. Nevertheless, the low percentages of assignable sequences point out the necessity for further work on gene characterization and functional determination within the acarid genera. Blast2GO top-hit species distribution revealed that 19 out of 29 top-hit species were arthropods (Fig. 2). With 21 914 hits, the mite *M. occidentalis* was the top-hit species followed by the deer tick *I. scapularis* (721 hits). This result was not unexpected since both species, as well as *D. gallinae*, belong to the super-order Parasitiformes and therefore share a close relationship. Furthermore, the genomes of both, *M. occidentalis* and *I. scapularis*, are completely sequenced and available at the common databases, which explains the high number of sequence similarities, especially to the mite *M. occidentalis*. Overall, 83·4% of homology hits (22 960 hits) were assigned to species belonging to the subclass Acari. There were also a number of *D. gallinae* sequences similar to non-arthropod organisms, corresponding to prokaryotic and eukaryotic key proteins of cell function, for example transcription, translation and elongation.

To gain knowledge of functional properties of *D. gallinae* transcripts, Gene Ontology (GO) terms were assigned to the dataset resulting in 15 482 mapped *D. gallinae* sequences (5·8%). In detail, 8034 of 231 657 singletons (3·5%), 18 of 56 contigs (32·1%) and 7430 of 35 751 isotigs (20·8%) were assigned to GO terms. Sequence assignment to the 'Cellular component' domain (seq filter = 10) yielded

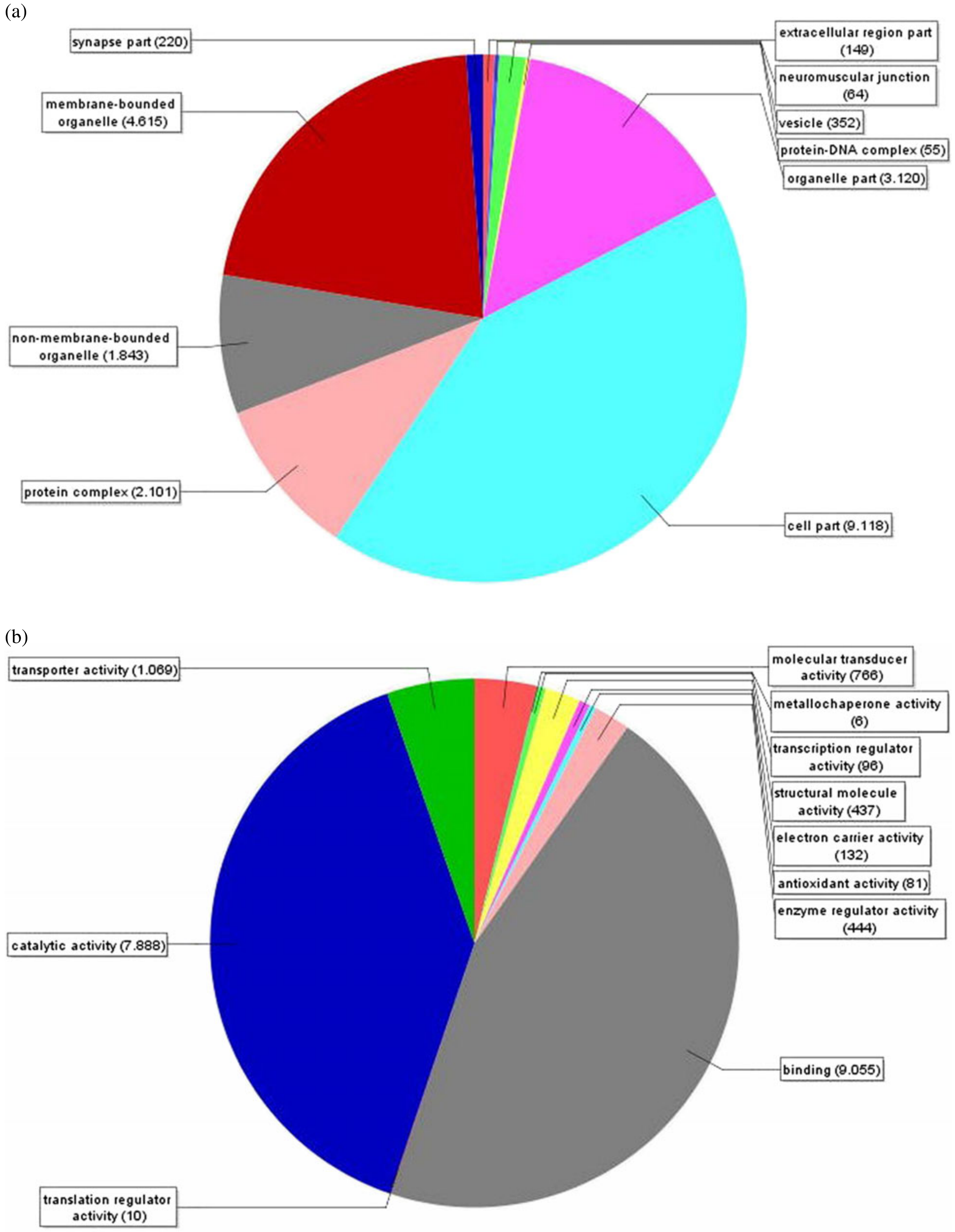

Fig. 3. (a–c) Gene Ontology term distribution: *D. gallinae* sequence annotation to GO categories. (a) Cellular component (seq filter 10%) on third-level subcategory; (b) Molecular function (seq filter 5%) on second-level subcategory; and (c) Biological process (seq filter 20%) on second-level subcategory. Numbers in parentheses represents the number of *D. gallinae* sequence hits.

21 637 hits on third-level subcategory (Fig. 3a). 'Molecular function' (seq filter = 5) and 'Biological process' (seq filter = 20) ontology distribution on second-level subcategory resulted in 19 984 (Fig. 3b) and 41 620 hits (Fig. 3c), respectively. More than three quarters (77·9%) of annotated 'Cellular

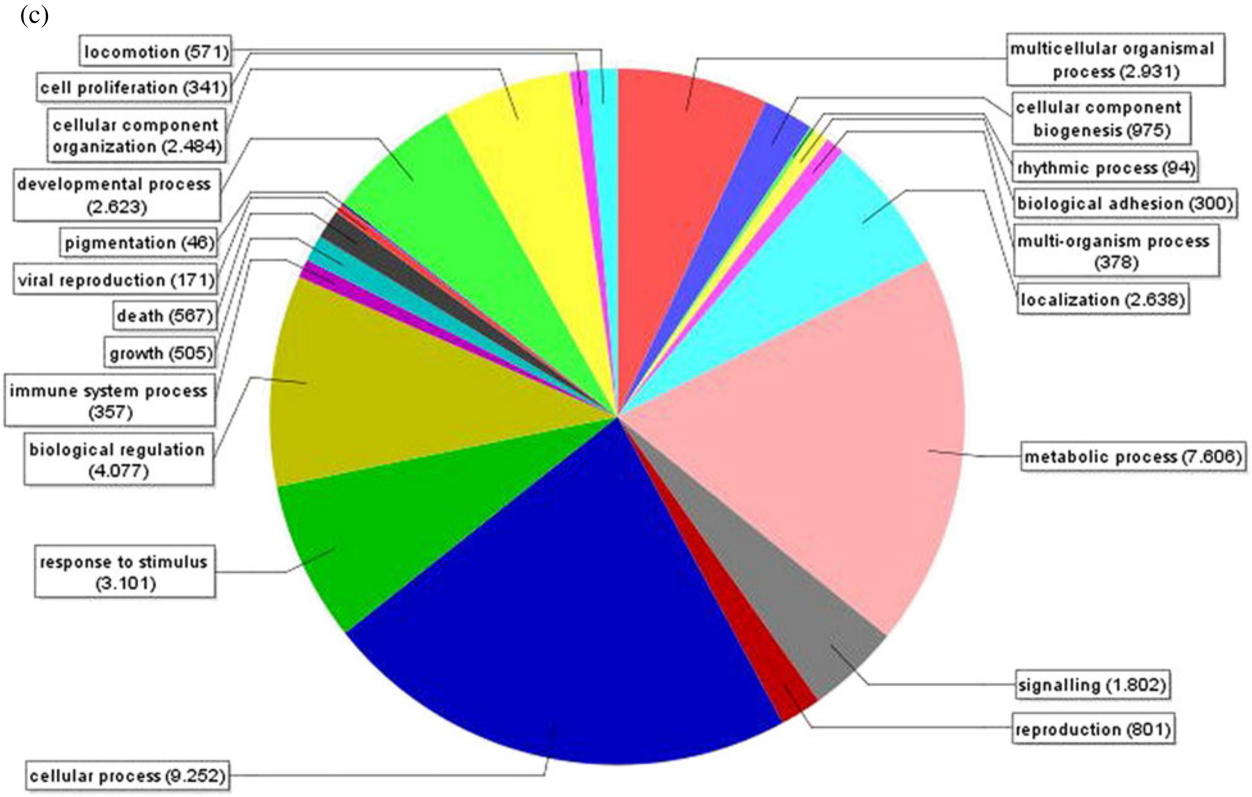

Fig. 3. (a–c) *continued*.

component' domain terms were allocated to the terms 'cell part' (9118 hits, 42·1%) followed by 'membrane-bound organelle' (4615 hits, 21·3%) and 'organelle part' (3120 hits, 14·4%). Within the GO domain 'Molecular function' the term 'binding' (9055 hits, 45·3%) followed by 'catalytic activity' (7888 hits, 39·5%) and 'transporter activity' (1069 hits, 5·4%) represented the majority of annotated GO terms. The term 'cellular process' comprised most of assigned *D. gallinae* transcripts (9252 hits, 22·2%) within the domain 'Biological process', followed by 'metabolic process' (7606 hits, 18·3%), 'response to stimulus' (3101 hits, 7·5%), and 'developmental process' (2623 hits, 6·3%). *Dermanyssus gallinae* is a transient ectoparasite which searches for its host at night whereas it hides in crevices during the day (Wood, 1917). Temperature as well as light signals are factors influencing the mite's activity (Harrison, 1963; Kirkwood, 1968). The latter plays an important role in circadian rhythm. This is mirrored by the GO category 'rhythmic process' within the domain 'Biological process', which was assigned to 0·2% of *D. gallinae* sequences (94 hits, cf. Fig. 3c). GO category 'locomotion' (571 hits) which includes locomotive behaviour and taxis, e.g. thermotaxis, which is known to direct mites' locomotion, was assigned to 1·4% of sequences.

Interestingly, the domain 'Biological process' exhibited 171 hits (0·4%) for the GO term 'viral reproduction', the process which includes infection of a host cell, replication of the viral genome, and assembly of progeny virus particles. In total, 62 *D. gallinae* sequences were assigned to this GO term. However, in BLASTx search the predominant part of these 62 sequences revealed non-viral proteins of eukaryotes as top-hits (almost half of the sequences matched to tick proteins; cf. Additional file 5 – in Online version only). Only two of the 62 sequences revealed a protein of possible viral origin as top-hit: One singleton (sequence number G9NSEKQ02I58T4) showed 77% similarity to the gag/env fusion protein isolated from *G. gallus* (*E*-value: 1·22E-27) and another singleton (sequence number G9NSEKQ02H1E7 V) resulted in 92% similarity to the envelope protein isolated from avian leukosis virus (*E*-value: 1·57E-23; cf. Additional file 5 – in Online version only). These sequences may derive from viral material ingested by the mite during a blood meal and subsequently included in transcriptome sequencing. However, it is also possible that viral genetic material has been integrated into the mite's genome since vector competence for the Avian Leukosis virus has already been described by Hilbrich (1978) and Hoffmann (1987).

For biological interpretation of higher-level systemic functions, the *D. gallinae* dataset was mapped to KEGG pathways. In total, 4580 sequences were assigned to 919 enzyme codes related to 127 KEGG pathways. Most pathways fell into the category 'metabolism', such as nucleotide and energy metabolism. The ten pathways most frequently represented by *D. gallinae* sequences are listed in Table 2. Astonishingly, the third most frequent (nitrogen

Table 2. Top 10 predicted KEGG pathways for *D. gallinae*

Pathway	Category	No. of sequences	No. of enzymes
Purine metabolism	Nucleotide metabolism	487	43
Pyrimidine metabolism	Nucleotide metabolism	192	25
Nitrogen metabolism	Energy metabolism	135	15
Methane metabolism	Energy metabolism	115	15
Oxidative phosphorylation	Energy metabolism	112	7
Valine, leucine and isoleucine degradation	Amino acid metabolism	109	19
T cell receptor signalling pathway	Immune system	106	2
Phenylalanine metabolism	Amino acid metabolism	99	13
Propanoate metabolism	Carbohydrate metabolism	99	18
Carbon fixation pathways in prokaryotes	Energy metabolism	96	17

Table 3. Top 20 predicted InterPro domains for *D. gallinae*

IPR accession	Domain name	Domain description	Occurrence
IPR001781	LIM	Zinc finger, LIM-type	565
IPR013783	No description	Immunoglobulin-like fold	498
IPR019734	TPR	Tetratricopeptide repeat	482
IPR003961	Fn3	Fibronectin, type III	454
IPR007087	Zf-C2H2	Zinc finger, C2H2	432
IPR000504	RRM	RNA recognition motif domain	415
IPR001680	No description	WD40 repeat	378
IPR001128	P450	Cytochrome P450	333
IPR002401	EP450I	Cytochrome P450, E-class, group I	325
IPR002126	CADHERIN	Cadherin	303
IPR008139	No description	Saposin B	291
IPR001611	LRR	Leucine-rich repeat	288
IPR019781	WD40	WD40 repeat, subgroup	281
IPR001452	SH3DOMAIN	Src homology-3 domain	270
IPR000477	RVT_1	Reverse transcriptase	265
IPR001806	RASTRNSFRMNG	Small GTPase superfamily	262
IPR007007	Ninjurin	Ninjurin	243
IPR007110	IG_LIKE	Immunoglobulin-like	231
IPR003591	No description	Leucine-rich repeat, typical subtype	229
IPR003599	No description	Immunoglobulin subtype	229

metabolism), fourth most frequent (methane metabolism), ninth most frequent (propanoate metabolism) and tenth most frequent (carbon fixation pathways in prokaryotes) pathways are known as energy metabolism pathways of prokaryotes. A similar result was also shown by Burgess *et al*. (2011) who annotated expressed sequence tags (ESTs) of *Psoroptes ovis* mites and found methane metabolism as the fifth most frequently occurring pathway. Mite sequence mapping to prokaryotic pathways can result from classification of common enzymes into such prokaryotic pathways. The enzyme peroxidase (ec:1.11.1.7), which is represented by 66 *D. gallinae* sequences and is suggested to be involved in the methane metabolism pathway catalyses only one reaction in this pathway but it is also known to occur in eukaryotes like animals and plants.

ESTScan was used for conceptual translation of all 267 464 *D. gallinae* sequences and resulted in 55 129 (20·6%) coding regions derived from 17 860 isotigs, 24 contigs and 37 245 singletons. The reason why only one fifth of all *D. gallinae* sequences were translated into amino acid sequences is explainable by the amount of sequences used to train ESTScan. Used sequences were all known sequences belonging to the superorder Parasitiformes. Subsequent InterPro sequence analysis, which is used for prediction of functional domains and motifs, identified 39 885 protein domains among the 55 129 identified coding regions. A number of 3723 different functional domains were identified. For each functional domain, one to 565 different *D. gallinae* sequences were assigned. Table 3 lists details on the top 20 predicted InterPro domains. The most frequently occurring domains correspond to zinc fingers e.g. of the LIM and zf-C2H2 domain. These domains are small protein motifs with finger-like protrusions that bind DNA, RNA, proteins and lipid substrates and are involved for example in gene transcription, protein-folding and building of cytoskeleton. Also very commonly found domains were the RNA recognition motif domain RRM as well as immunoglobulin-like folding motifs. Domains predicted for *D. gallinae* sequences were often involved in cell organizing functions such as protein expression, metabolism and development. These rather general functions were expected as the study aimed to cover the entire transcriptome of *D. gallinae*. The eighth most frequent domain was the P450 domain which corresponds to cytochrome P450 superfamily proteins. Additionally, KEGG analyses revealed enzymes involved in the pathways 'metabolism of xenobiotics by cytochrome P450' and 'drug metabolism – cytochrome P450', with 31 and 29 *D. gallinae* sequences corresponding to five and six enzymes, respectively, being involved in these pathways (data not shown). This might indicate the ability of poultry red mites to detoxify drugs or chemicals efficiently. However, cytochrome P450 proteins represent a large and diverse group of enzymes catalysing oxidation of organic substances like metabolic substances, e.g. lipids and steroidal hormones as well as xenobiotic substances, e.g. drugs (Danielson, 2002). This may underline the importance of these enzymes in metabolic processes of *D. gallinae*.

CONCLUSIONS

Genetic knowledge of poultry pests is essential to develop new strategies for control. With the current study dealing with the transcriptome analysis of the poultry red mite, the major parasitic pest of poultry, new insights in mite genomics are provided. Besides knowledge on the genetic level, essential data are provided for future research on new perspectives to control this parasitic mite by identifying possible drug targets or vaccine candidates. For instance, drug targets can be discovered by identification of essential and/or invertebrate specific transcripts followed by subsequent *in silico* prediction of interactions with drug compounds using appropriate databases such as BRENDA (Schomburg *et al*. 2004) or KEGG BRITE (Kanehisa *et al*. 2006). Besides extrapolation from vaccine studies performed with other macroparasites, potential *D. gallinae* vaccine candidates can be predicted by using bioinformatic tools supporting the identification of proteins which are directly (e.g. secreted proteins) or indirectly (e.g. membrane-bound gut proteins) available for the host's immune system.

SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0031182013001467.

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