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Tracing the evolutionary lineage of pattern recognition receptor homologues in vertebrates: An insight into reptilian immunity *via de novo* sequencing of the wall lizard splenic transcriptome

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ABSTRACT

Reptiles remain a deprived class in the area of genomic and molecular resources for the vertebrate classes. The transition of squamates from aquatic to terrestrial mode of life caused profound changes in their immune system to combat the altered variety of pathogens on land. The current study aims at delineating the evolution of defence mechanisms in wall lizard, *Hemidactylus flaviviridis*, by exploring its immunome. *De novo* sequencing of splenic transcriptome from wall lizard on the Illumina Hi-Seq platform generated 258,128 unique transcripts with an average GC content of 45%. Annotation of 555,557 and 6812 transcripts was carried out against NCBI (non-redundant database) and UniProt databases, respectively. The KEGG pathway annotation of transcripts classified them into 39 processes of six pathway function categories. A total of 3824 transcripts, involved in 23 immune-related pathways, were identified in the immune-relevant cluster built by harvesting the genes under KEGG pathways of immune system and immune diseases. Forty-two percent of the immune-relevant cluster was represented by pattern-recognition receptors (PRRs), of which the maximum number of transcripts was attributed to the Toll-like receptor (TLR) signalling pathway. Nine PRRs with potential full-length coding sequences were sorted for phylogenetic analysis and comparative domain analysis across the vertebrate lineage. They included DEC205/lymphocyte antigen 75 (ly75), nucleotide-binding oligomerisation domain-containing protein 1 (NOD1), NOD-like receptor family CARD domain-containing 3 (NLRC3), nucleotide-binding oligomerisation domain, leucine-rich repeat-containing X1 (NLRX1), DDX58/retinoic acid-inducible gene 1 (RIG-1), Toll-like receptor 3 (TLR3), TLR4, TLR5 and TLR7. From selection studies of these genes, we inferred positive selection for ly75, NOD1, RIG-1, TLR3 and TLR4. Apart from contributing to the scarce genomic resources available for reptiles and giving a broad scope for the immune-relevant pathways operative in wall lizards, this study will also pave way for evolutionary studies on comparative immunomics.

1. Introduction

The field of vertebrate "omics" has come a long way since the publication of the human genome in 2001 (IHGSC, 2001; Venter et al., 2001), courtesy of next-generation sequencing (NGS) technologies. NGS technologies not only provide high throughputs, but are also cost-effective and time-efficient (Hert et al., 2008). Their avid application in RNA sequencing has led to a massive transformation in the field of transcriptomics. Apart from precise detection and quantitation of transcripts, RNA sequencing also shows high reproducibility for both biological and technical replicates (Costa et al., 2010). It also enhances the probability of deriving greater functional information in a short span of time since assembly is easier with fewer repetitive elements and intronic sequences (Ekblom and Galindo, 2011). Until quite recently, mammals shared the limelight, with genome sequences of more than 40 species available online (Karolchik et al., 2014). Fortunately, with the execution of the Genome 10K project established in 2009, the initiative of sequencing the genomes of 10,000 vertebrates has gathered pace (G10KCOS, 2009).

The members of class reptilia are categorized as non-traditional model organisms. However, reptiles, being a key link between higher and lower vertebrates, occupy a critical position on the evolutionary timeline. These ectothermic amniotes depict the transition of physiological systems from ectothermic anamniotic fishes

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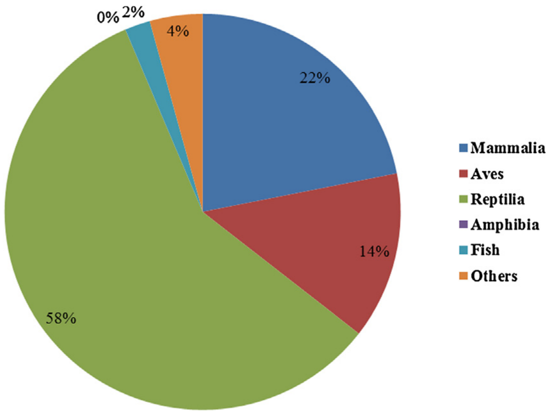

Fig. 1. Percentage distribution of BLAST hit species across the vertebrate classes.

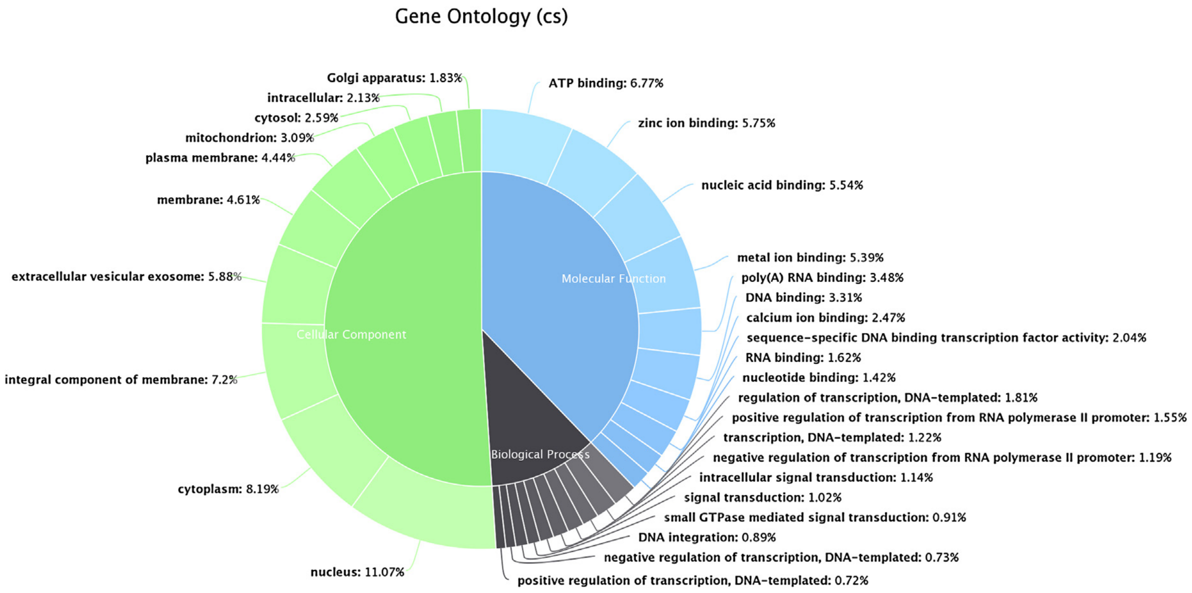

Fig. 2. Percentage distribution of functionally annotated transcripts across three GO categories biological processes, cellular components, and molecular functions.

and amphibians to endothermic amniotic birds and mammals (Zimmerman et al., 2010). Among the four orders listed under reptiles, squamata exhibited a complete switch to a terrestrial mode of life. It is apparent that this change must have been accompanied by reorganisation of energy resources for combatting the diverse changes on land. One of the challenges would have been the encountering of a new pathogen subset on land, thus seeding the onset of a unique makeup of reptilian immunity. As evident from a comparative study of mammalian and reptilian innate immunity, reptilian serum proteins had enhanced potency over the mammalian counterpart in the complement-mediated lysis of lyme disease spirochete *Borrelia burgdorferi* (Kuo et al., 2000). Another study attributed the resistance to *B. burgdorferi* infection in mice to the humoral response in the organism (Fikrig et al., 1990). This is indicative of a stronger innate immune system in reptiles as compared with that in mammals, which in turn might be replenishment for the presence of slow acquired immune responses in this class (Brames, 2007; Grey, 1963; Ingram and Molyneux, 1983; Kanakambika and Muthukkaruppan, 1972; Origgi et al., 2001; Pye et al., 2001). Reptilian immunity also features seasonal dependence (Tripathi et al., 2015; Zimmerman et al., 2010) and sexual dimorphism (Mondal and Rai, 1999). Although researchers have been trying to investigate various aspects of reptilian immunity since long, the information remains too limited to offer an integrated status to the functioning of the immune components in this class. The void is discernible while analyzing the immune components at a genetic or molecular level. The generation of additional genomic and molecular resources for this class of vertebrates will facilitate research in this direction.

Transcriptome data of several reptiles (Ullate Agote, 2014) and genome sequences of 12 species are available (Alföldi et al., 2011; Bar-Yaacov et al., 2013; Castoe et al., 2013; Shaffer et al., 2013; Pascual-Anaya and Zadissa, 2013; Song et al., 2015; St John et al., 2012; Vonk et al., 2013; Wan et al., 2014). Since most of this data has been generated over the past five years, analysis is at a very nascent stage. The major thrust is toward determining the phylogenetic position of the reptilian orders with respect to birds and mammals *via* comparative studies (Alföldi et al., 2011; Chiari et al., 2012; Crawford et al., 2012; Field et al., 2014; Hedges, 2012; St John et al., 2012; Tzika et al., 2011). Other studies concentrated on species-specific biological adaptations (Brykczynska et al., 2013; Miller et al., 2012; Schwartz et al., 2010; Wall et al., 2011; Yang et al., 2014), and comparison of GC content and sequence characteristics (repeats, transposons, microsatellites) across vertebrates (Figuet et al., 2015; Janes et al., 2011; Tollis and Boissinot, 2013). Apart from this, snake venomics is another field being explored *via* data generated through NGS platforms (Zelanis and Tashima, 2014). However, "omics" approaches have not yet been used extensively