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Research paper

Comparative transcriptomic analysis reveals the mechanism of leech environmental adaptation

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ABSTRACT

The medicinal leeches have been widely utilized in medical procedures for thousands of years. Recently, there were more and more transcriptomes of leech published online including the medicinal leech (*Hirudo medicinalis*) and some other leeches. However, leech's genetic backgrounds are still largely unknown. In this report, transcriptomes of three phylogenetically close leeches (*Poecilobdella javanica*, *Whitmania pigra*, and *Haemadipsa cavatuses*) were established by RNA-seq technique for studying their genetic mechanisms of environmental adaption. Over 110 million high-quality reads were generated and assembled into unique transcriptome (reads = 200 bp). 27,138 out of de novo assembled transcripts (41.77%) were assigned to one or more GO terms. Additionally, the transcripts were detected in 217 predicted KEGG pathways. The enriched genes were involved in protein metabolism, GPCRs and pathogen-resistant pathways. The results showed that the great variations existed in gene expression of olfactory transduction pathway among three leech species. The comparisons of leech species hinted at the underlying mechanism of leeches adapting well in various environments. Our study will provide useful rationales for future studies of leeches and other annelid species.

1. Introduction

Most medicinal leech species, such as *Poecilobdella javanica* and *Hirudo medicinalis*, are ectoparasites that feed on the blood of vertebrates including human (Yong and Yule, 2004). It serves as important model systems for understanding the structure, function, development, regeneration and repair of nervous systems (Macagno et al., 2010; Hibsh et al., 2015). They directly (Derganc and Zdravic, 1960; Gross and Apesos, 1992; Gideroglu et al., 2003; Herlin et al., 2017) or their secretions are used for medical procedures, such as clearing of pooled blood after certain surgical procedures and extracting of anticoagulants and antibacterial peptides (Seymour et al., 1990; Tasiemski et al., 2004; Zavalova et al., 2006). Recently, a new blood-sucking leech species *Haemadipsa cavatuses* was found living off bat blood exclusively in caves all lifelong (Yang, 2009). In addition, there are more than one group of leeches without hematophagic behaviors (Phillips and Siddall, 2009; Kvist et al., 2013) (e.g. *Whitmania pigra*), since it feeds only on small invertebrate such as insect and snail. It is well known that phylogenetically close species usually share similar living habitats, feeding strategies and common pathways in fundamental process (McCarroll et al., 2004). However, there were various natural habitats of three leech species with different feeding strategies (*W. pigra*, *P. javanica*, and *H. cavatuses*). We hypothesize that leeches, with diverse living habitats and feeding strategies, hold different patterns of gene expression adapt to their living environment. As a result, it is imperative to study the global transcriptional differences of these phylogenetically close organisms.

Rapid advances in genomic technologies, particularly those coupled

Abbreviations: RNA-seq, RNA sequencing; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes & Genome, GENES database; GPCR, G-protein-coupled receptor; *Poecilobdella javanica*, *P. javanica*; *Hirudo medicinalis*, *H. medicinalis*; *Haemadipsa cavatuses*, *H. cavatuses*; *Whitmania pigra*, *W. pigra*; COI, Cytochrome c Oxidase subunit I; CDS, coding sequence; COG, Clusters of Orthologous Groups; RPKM, per kilobase of exon per million mapped reads; DEG, differentially expressed gene; EST, expressed sequence tag

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to high-throughput sequencing technologies, have revolutionized our understanding of gene expression, gene regulation, and molecular mechanisms (Sankaran and Gallagher, 2013). Genomic and transcriptomic comparison of phylogenetically close organisms based on next-generation sequencing have been used for clarifying evolutionary relationships and environmental adaptability (Zhao et al., 2012; Zhao et al., 2014a, 2014b). To our knowledge, there were still less studies about the global gene expression profiles of leech species.

Although several leeches transcriptome were sequenced and analyzed by different methods (Macagno et al., 2010; Min et al., 2010; Kvist et al., 2014; Hibsh et al., 2015; Kvist et al., 2016; Siddall et al., 2016), we still need more sequenced data to analysis the gene function and genetic mechanism of environmental adaption, species evolution, etc. This led us to perform the high throughput paired-end RNA-seq (Mardis, 2008; Shendure and Ji, 2008) to depict the transcriptomes of three leech species. Furthermore, by comparative analysis, we attempt to study the mechanism that leech adapt to their diverse living environment with different habits. The data generated in the study will not only unravel the potential molecular mechanisms of leech evolution and adaptation, but also be of value for the future research of functional genomics.

2. Materials and methods

2.1. Animals and ethics

Three leech species were *P. javanica* (collected in forest stream in Lancang, Puer China: E99°93′85.68″, N22°56′18.02″), *W. pigra* (collected in pool water in Longyang, Baoshan China: E99°17′23.62″, N25°12′68.55″) and *H. cavatuses* (living in cave rock face or cave water, Mangshi, Ruili China: E97°85′84.68″, N24°01′85.15″), which collected from Yunnan Province of China (Fig. 1). Animal care and handling were conducted in accordance with the stipulations of Ethics Committee of Kunming University.

2.2. Species identifications

Specimens were identified with a stereomicroscope in previous studies (Yang, 1997; Wang, 2008; Yang, 2009). Species identifications were further verified with molecular sequences. With COI (Cytochrome c Oxidase subunit I) genes phylogenetic analysis, *H. cavatuses* was further genetic relationship with *P. javanica* that were phylogenetically close to *W. pigra* and *Hirudo medicinalis* (Fig. 2A). Phylogenetic analysis was performed by Maximum Likelihood method based on the Tamura-Nei model, which was complemented by the MEGA7 program (Tamura and Nei, 1993; Kumar et al., 2016). Substitution model were performed by Tamura-Nei model and bootstrap analysis, running 1000 bootstrap samples.

2.3. Tissue samples and RNA extraction

Tissues was used for RNA extraction from the whole body of these three species, before cleaned off their gastric tracts and the blood, which independently sampled from 10 to 20 adult leeches per species. Prepared tissues (approximately 200 mg) were preserved in liquid nitrogen for RNA extraction. Total RNAs were purified with RNA Easy Kit (QIANGEN, German) according to the manufacturers' instructions. RNA yields and the quality were measured by agarose gel electrophoresis and spectrophotometer (Thermo, USA). Equal amounts of total RNA (20 μg, 50.8 μg/ml) purified from each tissue were separately stored and mRNA was isolated with Oligo-dT Purist Kit (TaKaRa, Japan) according to the standard protocol.

2.4. Library construction and sequencing

Purified mRNA of leeches was added with fragmentation buffer for

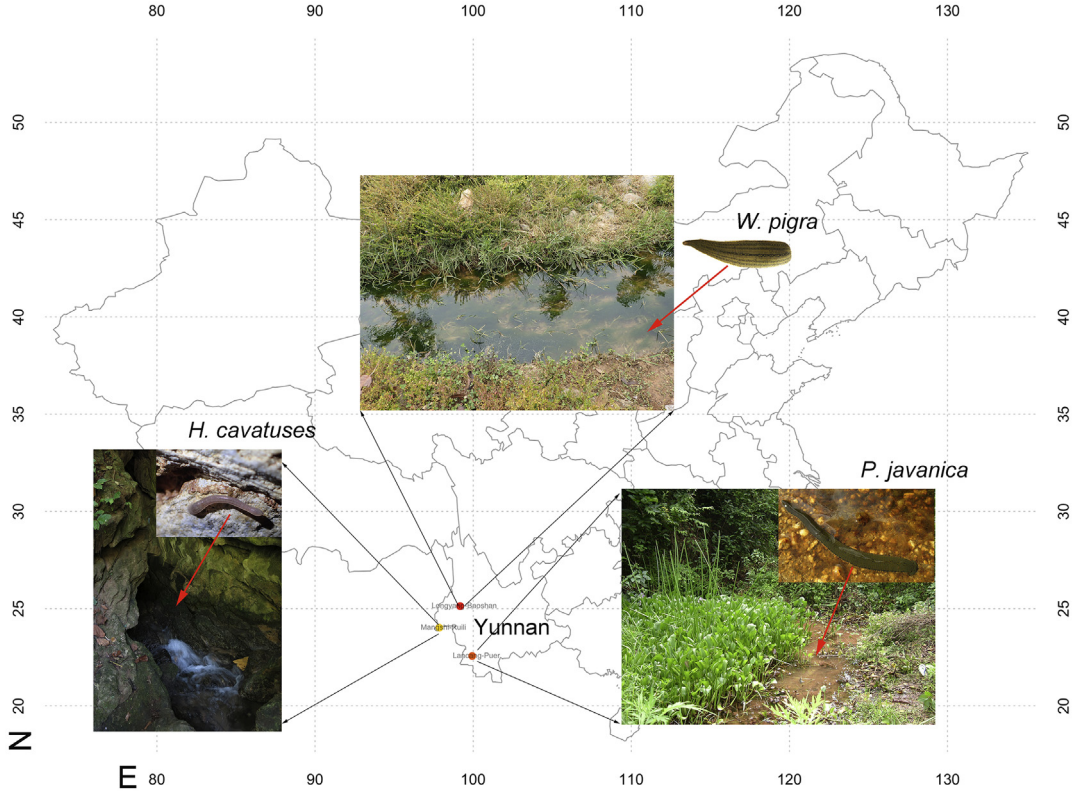

Fig. 1. The map of geographic location collected three species of leeches in China. Three leech species of *P. javanica*, *W. pigra*, and *H. cavatuses* were separately from Lancang, Longyang, and Mangshi in Yunnan Province of China. Specimens were identified by our previous studies.