Neotrop Entomol (2016) 45:397–403
DOI 10.1007/s13744-016-0388-8

ECOLOGY, BEHAVIOR AND BIONOMICS

Characterization of the Microbial Communities in the Ant Lion *Euroleon coreanus* (Okamoto) (Neuroptera: Myrmeleontidae)

JN Liu, TH Wang, QY Jia, XH Gao, H Wan, WY Sun, XL Yang, R Bao, JZ Liu, ZJ Yu

Key Lab of Animal Physiology, Biochemistry and Molecular Biology of Hebei Province, College of Life Sciences, Hebei Normal Univ, Shijiazhuang, People's Republic of China

Keywords
Illumina, microbiota, MiSeq sequencing, symbiont

Correspondence
JZ Liu and ZJ Yu, Key Lab of Animal Physiology, Biochemistry and Molecular Biology of Hebei Province, College of Life Sciences, Hebei Normal Univ, Shijiazhuang, 050024, People's Republic of China; liujingze@mail.hebtu.edu.cn; yzj116@163.com

Edited by Andrew Michel – Ohio State Univ

J N Liu, T H Wang and Q Y Jia contributed equally to this work.

Received 7 November 2015 and accepted 24 February 2016
Published online: 28 March 2016

Abstract

Euroleon coreanus (Okamoto) is widely distributed in China, and the larval stage can be treated as traditional Chinese medicine. However, the host-bacterium relationship remains unexplored, as there is a lack of knowledge on the microbial community of ant lions. Hence, in the current study, we explored the microbial community of the larval ant lion *E. coreanus* using Illumina MiSeq sequencing. Results indicated that a total of 10 phyla, 126 genera, and 145 species were characterized from the second instars of *E. coreanus*, and most of the microbes were classified in the phylum Proteobacteria. *Cronobacter muytjensii* was the most abundant species characterized in the whole body and gut of *E. coreanus*, and the unclassified species in the genera *Brevundimonas* and *Lactobacillus* were relatively more abundant in the head and carcass. In addition, no *Wolbachia*-like bacteria were detected, whereas bacteria like *Francisella tularensis* subsp. Holarctica OSU18 and unclassified *Rickettsiella* were first identified in ant lion *E. coreanus*.

Introduction

Ant lions are the semisedentary larval form of Myrmeleontidae, which represent the largest family of the order Neuroptera, with about 2000 species distributed throughout the world (Devetak *et al* 2010). Most of them are generalist predators that capture small passing arthropods by digging conical pits in sandy soil (Scharf *et al* 2009). The pit-trapping foraging strategy of the ant lion has received enormous research interests (Heinrich & Heinrich 1984, Devetak *et al* 2005, Beponis *et al* 2014), and their taxonomy (Wan & Wang 2003, Bao & Wang 2006), biology (Kitching 1984, Devetak *et al* 2013), and ecology (Scharf *et al* 2008, Rotkopf *et al* 2012) have also been extensively investigated.

The great pharmaceutical value of ant lions has been widely recognized in China. The earliest record of ant lion as traditional Chinese medicine formally appeared in the Compendium of Materia Medica (Ben Cao Gang Mu) of the Ming dynasty (AD 1590), and they were used to treat a variety of incurable diseases, including urinary tract stones, vasculitis, hypertension, otitis media, thrombosis, and other chronic diseases (Li *et al* 2013). Recently, two isoindoline alkaloids with potential pharmacological activities were characterized from the crude drug species of Myrmeleontidae ant lions (Nakatani *et al* 2006), and some volatile secretions, such as nerol, nostrenol, and 10-homonerol, have also been isolated from other ant lions (Baeckström *et al* 1989, Bergström *et al* 1992). Additionally, a paralytic polypeptide named ALBT-toxin was purified from the live ant lions *Myrmeleon bore* (Tjeder), and this toxin was proved to be produced by the bacterial isolates cultured from them (Matsuda *et al* 1995, Toshida *et al* 1999, Nishiwaki *et al* 2004). This raised an interesting argument that some of the pharmaceutical compounds may derive from the primary or secondary metabolites of the microbiota harbored by these ant lions. However, the host-bacterium relationship remains unexplored, as there is a lack of knowledge on ant lion-associated bacterial communities.

Dunn & Stabb (2005) performed culture-independent 16S rRNA gene sequence analysis of the bacteria associated with tissues of an ant lion, *Myrmeleon mobilis* (Hagen), and revealed that the main microbial species belongs to α-Proteobacteria with similarity to *Wolbachia* spp., and γ-Proteobacteria with similarity to the family *Enterobacteriaceae*. They also indicated the microbial community variation between ant lion species (Dunn & Stabb 2005). Hence, in the current study, we focused on the ant lion *Euroleon coreanus* (Okamoto), which is widely distributed in Korea, Mongolia, and North China, to explore its microbial community through Illumina MiSeq sequencing.

Material and Methods

Sample collection and tissue dissection

Ant lions were collected from the Xiaowutai National Natural Reserve Area (39°50′-40°07′N, 114°47′-115°30′E) of Hebei Province, North China. Representative adults were identified as *E. coreanus* according to the key described by Bao & Wang (2006). The second instar larvae with 0.5–0.7 cm in length were selected for genomic DNA isolation or tissue dissection. The ant lion samples were surface sterilized by immersion in absolute ethanol for 10 min, rinsed in sterile 1× phosphate-buffered saline (PBS) (260 mM NaCl, 10 mM Na_2HPO_4, 10 mM NaH_2PO_4, pH 7.2) for 5 min, followed by a second immersion in absolute ethanol for 5 min. Subsequently, the whole gut was isolated by a ventral incision and removal using forceps; head tissues were isolated by removing the head from the body using a scalpel blade. Body tissue was defined as the remaining tissue after gut and head removal. The whole ant lions (three ant lions) and the dissected tissues (from three ant lions) were immediately snap-frozen in liquid nitrogen and further used.

Genomic DNA extraction, PCR amplification, and amplicon quantification

The genomic DNA was purified using the DNeasy Tissue Kit (Qiagen, Germany), according to the manufacturer's instructions. The extracted genomic DNA was measured with Nanodrop (Thermo Fisher Scientific, US) and electrophoresed in 1% (*w*/*v*) agarose gel. The V4–V5 region of the bacteria 16S ribosomal RNA gene was amplified by PCR (95°C for 2 min, followed by 25 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s and a final extension at 72°C for 5 min) using primers 515F (5′-barcode-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTR AGTTT-3′), where barcode is an eight-base sequence unique to each sample (Zhou *et al* 2011). PCR reactions were performed in triplicate 20 μL mixture containing 4 μL of 5× FastPfu buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA), according to the manufacturer's instructions and quantified using QuantiFluor™-ST (Promega, USA).

MiSeq library construction and sequencing

Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 250) on an Illumina MiSeq platform according to the standard protocols. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP051666).

Raw fastq files were demultiplexed and quality-filtered using QIIME (version 1.17) with the following criteria: (i) 250-bp reads were truncated at any site receiving an average quality score <20 over a 10-bp sliding window, discarding the truncated reads that were shorter than 50 bp; (ii) exact barcode matching, two nucleotide mismatches in primer matching, and reads containing ambiguous characters were removed; and (iii) only sequences that overlap longer than 10 bp were assembled according to their overlap sequence. Reads that could not be assembled were discarded.

Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/), and chimeric sequences were identified and removed using UCHIME (version 4.2.40 http://drive5.com/usearch/manual/uchimealgo.heml). The phylogenetic affiliation of each 16S rRNA gene sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the Silva (SSU115) 16S rRNA database using a confidence threshold of 70% (Amato *et al* 2013). Samples were first clustered according to beta-diversity matrices, followed by unweighted pair group method with arithmetic mean (UPGMA) clustering based on the UniFrac distance matrix, and heat map was generated using a hierarchical clustering algorithm (Jiang *et al* 2013).

Results

A total of 74,918 valid sequences, 72,880 trimmed reads, and 363 OTUs were obtained from the four samples through Illumina MiSeq sequencing (Online Supplementary Material—Table S1). Among them, 11,524 reads clustered in 49 OTUs from the whole body of *E. coreanus* (Ec_all), and 28,112 reads clustered in 123 OTUs and 20,422 reads in 97 OTUs were produced from the gut (Ec_gut) and head (Ec_head) of the ant lion, respectively. After removing the gut and head, the carcass (Ec_other) of the ant lion yielded the remaining 12,822 reads which were classified into 94 OTUs (Table 1).

The rarefaction curves showed that all samples sequencings, except the whole body, have approached the saturation plateau (Online Supplementary Material—Fig S1), which indicated that the sequencing depth of these samples

Table 1 Statistics of sample sequences obtained from the ant lion *Euroleon coreanus*.

Sample	Reads	Label: 0.97				
		OTU	Ace	Chao	Shannon	Simpson
Ec_all	11,524	49	83 (63,133)	75 (58,122)	0.47 (0.45, 0.5)	0.8423 (0.8333, 0.8514)
Ec_gut	28,112	123	125 (123,131)	124 (123,132)	2.5 (2.48, 2.52)	0.1574 (0.1546, 0.1602)
Ec_head	20,422	97	108 (101,125)	106 (100,126)	1.98 (1.96, 2)	0.2464 (0.2417, 0.2511)
Ec_other	12,822	94	95 (94,101)	95 (94,102)	2.48 (2.46, 2.51)	0.1436 (0.1404, 0.1468)

represented the microbial communities very well. By analyzing the Shannon-Wiener index (Wang *et al* 2012), the curves tended to level off (Online Supplementary Material—Fig S2). The number of OTUs was obtained at 3% dissimilarity, and out of the 363 OTUs, a total of 160 OTUs were detected unique in all the four samples (Fig 1).

Taxonomic abundance of the obtained sequences was summarized at the phylum, genus, and species levels. At the phylum level, a total of 10 phyla were identified from all the four samples. Among these identified microbes, Proteobacteria were the most abundant in the whole body (97.0%), gut (68.6%), head (64.3%), and the carcass (68.2%) (Fig 2a). Firmicutes was also abundant in the gut (19.3%), head (32.5%), and the carcass (28.7%), but relatively low in the whole body (0.3%). Deinococcus-Thermus was obviously high in the gut (8.9%), whereas low in the other samples. There was no obvious difference in the distribution of Actinobacteria in the gut (2.7%), head (2.3%), and the carcass (2.1%), whereas it was relatively low in the whole body (0.6%). Acidobacteria was not detected in the gut, whereas Fusobacteria and Spirochaetae were only detected in the gut. Tenericutes was exclusively associated with the carcass of *E. coreanus* (Fig 2a).

Microbes from all four samples were assigned into 126 genera (Online Supplementary Material—Table S2). The gut of the ant lion harbored the most diversity of microbes, and

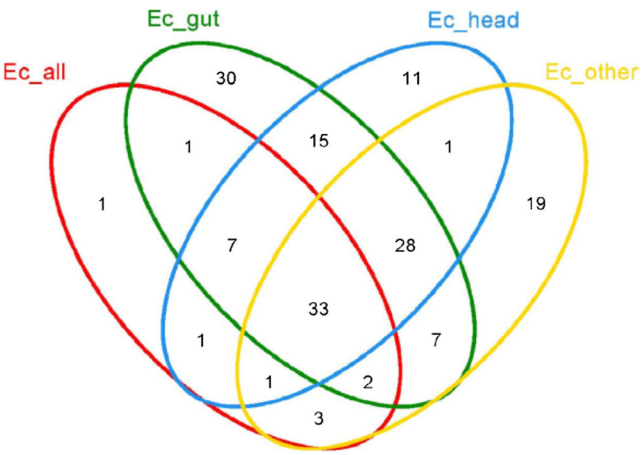

Fig 1 The different distribution of the unique OTUs clustered in all the four tested samples of ant lion *Euroleon coreanus*.

the most abundant microbes characterized in the whole body were *Cronobacter*, which were widely distributed in all tissues of *E. coreanus* ant lion. *Brevundimonas*, *Lactobacillus*, and *Alcaligenes* were widely distributed in the head and carcass, and a large amount of *Lactococcus* was identified in the gut and head, whereas unclassified *Enterobacteriaceae* was harbored in the gut (Fig 2b).

To further explore the diversity of microbes associated with the ant lion *E. coreanus*, the relative abundance of the characterized microbial species were summarized (Online Supplementary Material—Table S3). *Cronobacter muytjensii* was the most abundant species characterized in the whole body and gut of *E. coreanus*, followed by unclassified species of *Pantoea* and *Acinetobacter calcoaceticus* in the whole body, and *Thermus scotoductus* and unclassified *Lactobacillus* in the gut. In the head and carcass of the ant lion, unclassified species in the genera *Brevundimonas* and *Lactobacillus* were relatively more abundant, followed by *Alcaligenes faecalis* and unclassified *Lactococcus* in the head, and *C. muytjensii* and unclassified *Rickettsiella* in the carcass (Table 2). It is worth to note that *Francisella tularensis* subsp. Holarctica OSU18 was first identified from the ant lion *E. coreanus*.

According to hierarchical clustering analysis, differences of the microbial composition among all tested samples were calculated using unweighted pair group method with arithmetic mean. Results indicated that the microbial community in the head of the ant lion was similar with that characterized in the carcass, whereas the microbes identified from the gut were more similar with that from the whole body of *E. coreanus* ant lion (Fig 3). Out of the 126 identified genera, the top 100 genera were clustered according to the similarity detected between tested samples (Fig 3). Most of the characterized microbes were harbored in the gut.

Discussion

Data on the microbial community of ant lion larvae are rare, and the only study on the characterization of the microbes in the ant lion *M. mobilis* (Hagen) indicated that the microbiota was qualitatively similar throughout the three larval stages, but that the microbial communities may vary between ant

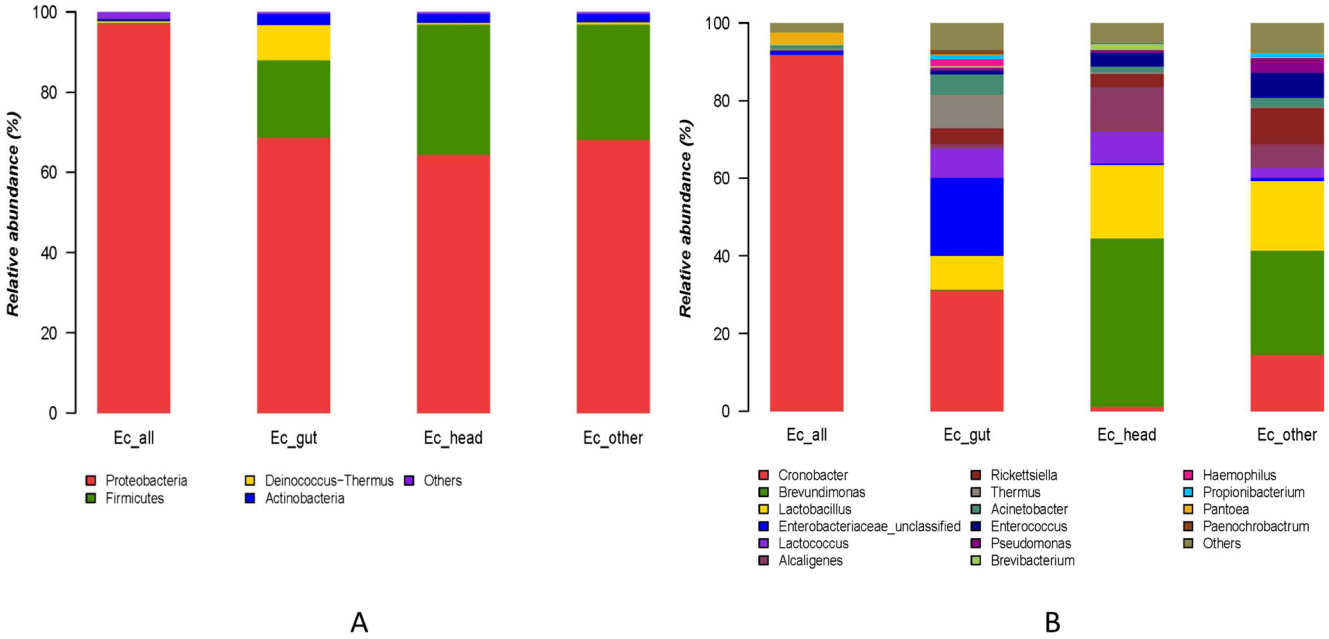

Fig 2 Relative abundance of the characterized microbes at phylum and genus level per sample of ant lion *Euroleon coreanus* (**A** Phylum level. **B** Genus level).

lion species or between different geographic populations (Dunn & Stabb 2005).

We demonstrated a total of 10 phyla, 126 genera, and 145 species were characterized from the second instars of the ant lion *E. coreanus*, and most of the microbes belonged to Proteobacteria, followed by Deinococcus-Thermus, Firmicutes, and Actinobacteria. When compared to the microbiota from *M. mobilis*, which was dominated by α- and γ-Proteobacteria bacteria (Dunn & Stabb 2005), a slightly more diverse microbial community, including the β-Proteobacteria, Deinococci, Bacilli, and Actinobacteria, was characterized from *E. coreanus*. However, no *Wolbachia*-like bacteria were detected from *E. coreanus*, as observed in ant lion larvae from Japan (Egami *et al* 2009), which indicated that *Wolbachia* are not commonly associated with ant lions in Asia. Although *Wolbachia* are ubiquitously found in diverse insects and also regarded as essential for host's growth and reproduction (Nikoh *et al* 2014), the association between *Wolbachia* and ant lions remains unknown (Dunn & Stabb 2005). In addition, some bacteria like *F. tularensis* subsp. Holarctica OSU18 and unclassified *Rickettsiella* were first identified in the ant lion *E. coreanus*.

The morphology and functions of the discontinuous gut in ant lions have attracted a great interest from researchers (Van Zyl *et al* 1997, Lipovšek *et al* 2012), whereas the microbial community harbored in the gut was presumed to be relatively simple (Dunn & Stabb 2005). However, in the current study, the gut of *E. coreanus* was proved to contain more diversity of microbes than the head and the carcass (Fig 2), with the γ-Proteobacteria *C. muytjensii* (formerly called *Enterobacter sakazakii*), as the most abundant symbiont. *Cronobacter* spp. have been isolated externally or internally (mostly in intestinal tracts) from several species of flies, such as the Mexican fruit fly *Anastrepha ludens* (Loew) and the stable fly *Stomoxys calcitrans* (Linnaeus) (Pava-Ripoll *et al* 2012). Previous studies indicated that in the gut of *M. mobilis* (Dunn & Stabb 2005) and *M. bore* (Nishiwaki *et al* 2004), *Enterobacter aerogenes* and *Bacillus cereus* were the most common bacterial associates, respectively, and these bacteria were regarded as the source of insecticidal or bactericidal peptides. *Enterobacter aerogenes* and *B. cereus* were not found in the ant lion *E. coreanus*, but an unclassified *Pantoea* species was characterized in ant lion *E. coreanus*, which has been found to protect the host from colonization by other microorganisms by producing antimicrobial compounds (Dillon & Charnley 1995).

The microbiota may also differ from tissue to tissue, as we demonstrated by the differences in the microbiota associated with the head, gut, and carcass of the ant lion *E. coreanus* (Fig 3). A relatively low amount of *C. muytjensii* was characterized from the head of *E. coreanus*, a tissue mostly colonized by an unclassified *Brevundimonas* species. *Brevundimonas* have been characterized from many other insects, including the heads of sharpshooters (Gai *et al* 2011) and gut of soybean aphid (Bansal *et al* 2014) and other insects (Hu *et al* 2014, Merville *et al* 2013), whereas its function remains to be explored. *Lactococcus* and *Lactobacillus* bacteria are frequently found in animal guts, and they usually help the host to produce amino acids and vitamins that they do not synthesize themselves (Reeson *et al* 2003). In the current study, the relatively high abundances of *Lactococcus* and *Lactobacillus* in the gut and head of *E. coreanus* may be associated with

Table 2 Relative abundance of the main microbes identified in the ant lion *Euroleon coreanus*.

Phylum	Class	Order	Family	Genus	Species	Relative abundance (%)			
						Ec-all	Ec-gut	Ec-head	Ec-other
Proteobacteria	γ-Proteobacteria	*Enterobacteriales*	*Enterobacteriaceae*	*Cronobacter*	*Cronobacter muytjensii*	91.71	31.03	1.23	14.56
				Pantoea	–	2.92	0.19	0.01	0.12
		Legionellales	*Coxiellaceae*	*Rickettsiella*	–	0	4.20	3.49	9.24
		Pasteurellales	*Pasteurellaceae*	*Haemophilus*	*Haemophilus parainfluenzae*	0	1.73	0	0
		Pseudomonadales	*Moraxellaceae*	*Acinetobacter*	–	0.01	0.72	0.89	1.84
				Acinetobacter	*Acinetobacter calcoaceticus*	0.80	4.59	0.57	0.41
			Pseudomonadaceae	*Pseudomonas*	–	0.03	0.44	0.31	2.59
				Pseudomonas	*Pseudomonas citronellolis*	0.01	0.24	0.06	0.91
		Xanthomonadales	*Xanthomonadaceae*	*Silanimonas*	Uncultured *Xanthomonadales* bacterium	0	0.36	0.17	0.05
		Thiotrichales	*Francisellaceae*	*Francisella*	*Francisella tularensis* subsp. Holarctica OSU18	0.03	0.11	0.61	0.14
	β-Proteobacteria	*Burkholderiales*	*Comamonadaceae*	*Diaphorobacter*	–	0.02	0.48	0.16	0.34
			Alcaligenaceae	*Alcaligenes*	*Alcaligenes faecalis*	0.11	1.05	11.44	6.21
	α-Proteobacteria	*Rhizobiales*	*Brucellaceae*	*Paenochrobactrum*	Uncultured bacterium	0.03	1.05	0.11	0.02
			Rhizobiaceae	*Rhizobium*	–	0	0.14	0.07	0.85
		Caulobacterales	*Caulobacteraceae*	*Brevundimonas*	–	0.06	0.31	43.36	26.74
Deinococcus-Thermus	Deinococci	*Thermales*	*Thermaceae*	*Thermus*	*Thermus scotoductus*	0.37	8.64	0.41	0.36
				Deinococcus	Uncultured organism	0	0.24	0.02	0.10
Firmicutes	Bacilli	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	–	0	8.62	18.83	17.98
				Pediococcus	–	0	0.12	0.49	0.41
			Leuconostocaceae	*Weissella*	*Weissella koreensis* KACC 15510	0	0.13	0.65	0.24
			Streptococcaceae	*Lactococcus*	–	0.02	7.61	8.24	2.53
			Carnobacteriaceae	*Dolosigranulum*	Uncultured organism	0	0.11	0	0
		Bacillales	*Staphylococcaceae*	*Staphylococcus*	*Staphylococcus aureus* subsp. Aureus ST228	0.05	0.67	0.3	0.3
			Paenibacillaceae	*Oxalophagus*	Uncultured bacterium	0.04	0.49	0.01	0.07
Actinobacteria	Actinobacteria	*Propionibacteriales*	*Propionibacteriaceae*	*Propionibacterium*	*Propionibacterium acnes*	0.03	1.03	0.07	0.79
		Micrococcales	*Brevibacteriaceae*	*Brevibacterium*	–	0.3	0.52	1.61	0.29
		Corynebacteriales	*Dietziaceae*	*Dietzia*	–	0.01	0.32	0.33	0.13

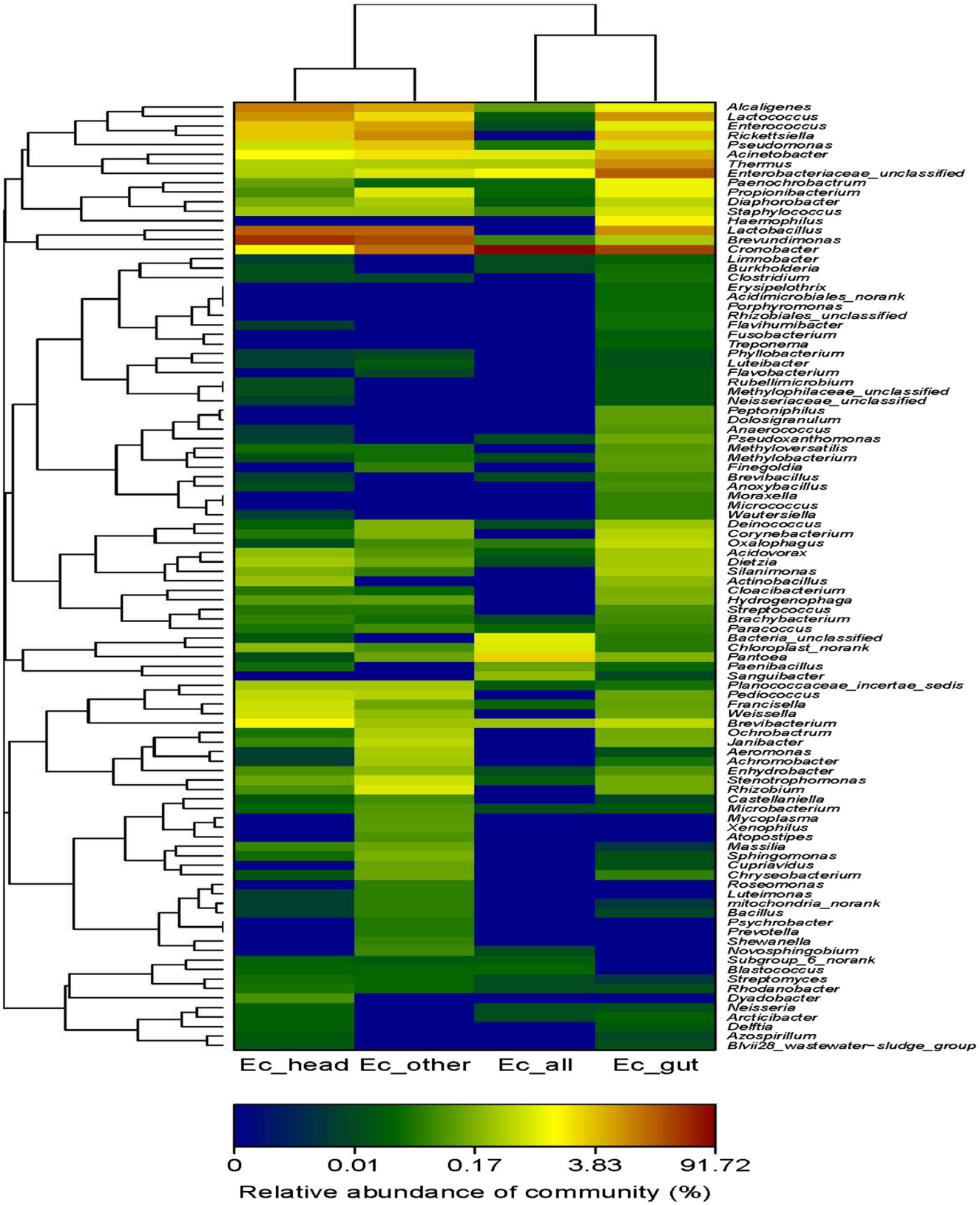

Fig 3 Heatmap analysis on the top 100 genera of the microbes identified in the ant lion *Euroleon coreanus*.

its special feeding behavior and gut physiology. Further studies are now required to clarify the interactions between the colonized bacteria, the ant lion host, and the great pharmaceutical potential of the ant lion.

Acknowledgments This work was supported by the National Natural Science Foundation of China (31400342, 31272372), Natural Science Foundation of Hebei (C2015205124), Natural Science Research Programs of Educational Department of Hebei Province (BJ2016032), and Science Foundation of Hebei Normal University (L2012Z05, L2011B13).

Electronic supplementary material The online version of this article (doi:10.1007/s13744-016-0388-8) contains supplementary material, which is available to authorized users.

References

Amato KR, Yeoman CJ, Kent A, Righini N, Carbonero F, Estrada A, Gaskins HR, Stumpf RM, Yildirim S, Torralba M, Gillis M, Wilson BA, Nelson KE, White BA, Leigh SR (2013) Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. ISME J 7: 1344–1353

Baeckström P, Bergström G, Björkling F, Hui-zuh H, Högberg HE, Jacobsson U, Li GQ, Löfqvist J, Norin T, Wassgren AB (1989) Structures, absolute configurations, and syntheses of volatile signals from three sympatric ant-lion species, *Eurolen nostras*, *Grocus bore*, and *Myrmelen formicarius* (Neuroptera: Myrmeleontidae). J Chem Ecol 15:60–80

Bansal R, Mian RMA, Michel AP (2014) Microbiome diversity of *Aphis glycines* with extensive superinfection in native and invasive populations. Environ Microbiol Rep 6:57–69

Bao R, Wang XL (2006) A review of the species of *Euroleon* (Neuroptera: Myrmeleontidae) from China. Zootaxa 1375:51–57

Beponis LM, O'Dea RE, Ohl VA, Ryan MP, Backwell PR, Binning SA, Haff TM (2014) Cleaning up after a meal: the consequences of prey disposal for pit-building antlion larvae. Ethology 120(9):873–880

Bergström G, Wassgren AB, Högberg HE, Hedenström E, Hefetz A, Simon D, Ohlsson T, Löfqvist J (1992) Species-specific, two-component, volatile signals in two sympatric ant-loin species: *Synclysis baetica* and *Acanthaclisis occitanica* (Neuroptera, Myrmeleontidae). J Chem Ecol 18:1177–1188

Devetak D, Špernjak A, JanžekoviČ F (2005) Substrate particle size affects pit building decision and pit size in the antlion larvae *Euroleon nostras* (Neuroptera: Myrmeleontidae). Physiol Entomol 30(2):158–163

Devetak D, Lipovšek S, Pabst MA (2010) Morphology and biology of the antlion *Myrmeleon yemenicus* Hölzel, 2002 (Neuroptera, Myrmeleontidae). Zootaxa 2531:48–56

Devetak D, Klokocovnik V, Lipovsek S, Bock E, Leitinger G (2013) Larval morphology of the antlion *Myrmecaelurus trigrammus* (Pallas, 1771) (Neuroptera, Myrmeleontidae), with notes on larval biology. Zootaxa 3641(4):491–500

Dillon RJ, Charnley AK (1995) Chemical barriers to gut infection in the desert locust: In vivo production of antimicrobial phenols associated with the bacterium *Pantoea agglomerans*. J Invertebr Pathol 66:72–75

Dunn AK, Stabb EV (2005) Culture-independent characterization of the microbiota of the ant lion *Myrmeleon mobilis* (Neuroptera: Myrmeleontidae). Appl Environ Microbiol 71(12):8784–8794

Egami I, Iiyama K, Zhang P, Chieda Y, Ino N, Hasegawa K, Lee JM, Kusakabe T, Yasunaga-Aok C, Shimizu S (2009) Insecticidal bacterium isolated from an ant lion larva from Munakata, Japan. J Appl Entomol 133:117–124

Gai CS, Dini-Andreote F, Andreote FD, Lopes JRS, Araújo WL (2011) Endophytic bacteria associated to sharpshooters (Hemiptera: Cicadellidae), insect vectors of *Xylella fastidiosa* subsp. pauca. J Plant Pathol Microbiol 2:109

Heinrich B, Heinrich MJE (1984) The pit-trapping foraging strategy of the ant lion, *Myrmeleon immaculatus* DeGeer (Neuroptera: Myrmeleontidae). Behav Ecol Sociobiol 14(2):151–160

Hu X, Yu JM, Wang CY, Chen H (2014) Cellulolytic bacteria associated with the gut of *Dendroctonus armandi* larvae (Coleoptera: Curculionidae: Scolytinae). Forests 5:455–465

Jiang XT, Peng X, Deng GH, Sheng HF, Wang Y, Zhou HW, Tam NFY (2013) Illumina sequencing of 16S rRNA tag revealed spatial variations of bacterial communities in a mangrove wetland. Microb Ecol 66:96–104

Kitching RL (1984) Some biological and physical determinants of pit size in larvae of *Myrmeleon pictifrons* Gerstaecker (Neuroptera: Myrmeleontidae). Aust J Entomol 23(3):179–184

Li JD, Huang LY, Qu XB (2013) Chinese medicine animal journal, 2nd edn. Science and Technology Publishing House of Fujian, Fujian, p 103

Lipovšek S, Letofsky-Papst I, Hofer F, Pabst MA, Devetak D (2012) Application of analytical electron microscopic methods to investigate the function of spherites in the midgut of the larval antlion *Euroleon nostras* (Neuroptera: Myrmeleontidae). Microsc Res Tech 75(4):397–407

Matsuda K, Suzuki H, Nakanishi F, Shio K, Komai K, Nishimura K (1995) Purification and characterization of a paralytic polypeptide from larvae of *Myrmeleon bore*. Biochem Biophys Res Commun 215(1):167–171

Merville A, Venner S, Henri H, Vallier A, Menu F, Vavre F, Hedd A, Bel-Venner MC (2013) Endosymbiont diversity among sibling weevil species competing for the same resource. BMC Evol Biol 13:28

Nakatani T, Nishimura E, Noda N (2006) Two isoindoline alkaloids from the crude drug, the ant lion (the larvae of Myrmeleontidae species). J Nat Med 60(3):261–263

Nikoh N, Hosokawa T, Moriyama M, Oshima K, Hattori M, Fukatsu T (2014) Evolutionary origin of insect—*Wolbachia* nutritional mutualism. Proc Natl Acad Sci U S A 111(28):10257–10262

Nishiwaki H, Ito K, Otsuki K, Yamamoto H, Komai K, Matsuda K (2004) Purification and functional characterization of insecticidal sphingomyelinase C produced by *Bacillus cereus*. Eur J Biochem 271: 601–606

Pava-Ripoll M, Pearson REG, Miller AK, Ziobro GC (2012) Prevalence and relative risk of *Cronobacter* spp., *Salmonella* spp., and *Listeria* monocytogenes associated with the body surfaces and guts of individual filth flies. Appl Environ Microbiol 78:7891–7902

Reeson AF, Jankovic T, Kasper ML, Rogers S, Austin AD (2003) Application of 16S rDNA-DGGE to examine the microbial ecology associated with a social wasp *Vespula germanica*. Insect Mol Biol 12:85–91

Rotkopf R, Barkae ED, Bar-Hanin E, Alcalay Y, Ovadia O (2012) Multi-axis niche examination of ecological specialization: responses to heat, desiccation and starvation stress in two species of pit-building antlions. PLoS One 7(11):e50884

Scharf I, Hollender Y, Subach A, Ovadia O (2008) Effect of spatial pattern and microhabitat on pit construction and relocation in *Myrmeleon hyalinus* (Neuroptera: Myrmeleontidae) larvae. Ecol Entomol 33(3):337–345

Scharf I, Filin I, Ovadia O (2009) A trade-off between growth and starvation endurance in a pit-building antlion. Oecologia 160(3):453–460

Toshida N, Sugama H, Gotoh S, Matsuda K, Nishimura K, Komai K (1999) Detection of ALMB-toxin in the larval body of *Myrmeleon bore* by anti-N-terminus peptide antibodies. Biosci Biotechnol Biochem 63:232–234

Van Zyl A, Van Der Westhuizen MC, Van Der Linde TDK (1997) Utilisation of prey by antlion larvae (Neuroptera: Myrmeleontidae) in terms of energy and nutrients. J Insect Physiol 43(7):605–613

Wan X, Wang XL (2003) Surveys of the taxonomy on Myrmeleontidae. Entomol Knowl 40(3):218–223

Wang Y, Sheng HF, He Y, Wu JY, Jiang YX, Tam NFY, Zhou HW (2012) Comparison of the levels of bacterial diversity in freshwater, intertidal wetland, and marine sediments by using millions of illumina tags. Appl Environ Microbiol 78:8264–8271

Zhou J, Wu L, Deng Y, Zhi X, Jiang YH, Tu QC, Xie JP, Nostrand JDV, He ZL, Yang YF (2011) Reproducibility and quantitation of amplicon sequencing-based detection. ISME J 5:1303–1313