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Original Research

Gut Microbiota and Accumulation of Heavy Metals: A New Study of Water Scorpions (Hemiptera: Nepidae)

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Abstract

This is a multidisciplinary study that examines the accumulation of heavy metals and gut microbiota in a water scorpion insect for the first time. Water scorpions can tolerate pollution to a certain extent. This investigation aimed to reveal some bacterial subspecies with biotechnological importance included within the gut structures of a water scorpion. The amount and purity of the isolated DNA was determined fluorometrically with 16s rRNA gene and amplified for use in species determination. The detected bacterial subspecies were as follows; list of bacteria in *Nepa* spp. gut microbiota. Additionally, some heavy metals were detected and evaluated. The correlation of heavy metals and bacteria, which are biotic elements of gut microbiota, in terms of environmental sustainability in indicator species was discussed. Indictor hemipterans in wetlands play important roles as ecosystem engineers to improve self-purification and promote elemental cycling. Therefore, our results will affect future studies about ecology.

Keywords: aquatic insects, freshwater, heavy metals, indicator species, metagenomics analysis

Introduction

In ecosystems, biotic impact is expected to increase worldwide due to human action [1]. Human activities negatively influence ecosystems [2]; these actions are major causes of stress in natural ecosystems [3]. Even if inland-protected areas previously focused on terrestrial ecosystems, multiple studies focus on protecting freshwater biodiversity and water ecosystems [4]. Some aquatic populations affect aquatic resources [5]. Especially macroinvertebrates comprise a biotic factor in aquatic areas. Macroinvertebrates (mostly insects) are important groups for water quality monitoring about the effect of pollution [6]. Insects provide services for our societies, which are essential for a sustainable future. The absence of insects means the collapse of food production [7]. Aquatic insects are considered model organisms for analyzing freshwater ecosystems due to their high abundance, large biomass and rapid colonization of habitats [8]. Freshwater ecosystems were studied for better understanding of the relationship between biotic and abiotic factors. Biotic factors in freshwater systems include microbiology, which are crucial [9].

Environmental or chemical pollutants can also impact gut bacterial communities (Lindell, et al. 2022).

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Moreover, gut bacterial communities play crucial roles in insect susceptibility to infections and insecticides [10]. The use of metagenomics has become a common technique to explore the effects of pollution in some ecosystems by evaluating changes in microbial communities and microbiome [11]. Gut bacteria are known to have multifarious roles in food digestion and nutrition and confer protection against pathogens and parasites. The interactions between gut bacteria and microbiome are unknown [12]. This study is important from the viewpoint of other intestine research. Many researchers used insect intestines to address fundamental questions about stem cell functions during tissue maintenance and regeneration because of the cellular and genetic similarities of insects to the mammalian intestine, and their relevance as a target for the control of insect pests and disease vectors [13]. In this context, identifying the microbiome of aquatic Hemiptera was our primary purpose. Biological indices are based on the richness of the aquatic community [14]. Aquatic insects help to indicate the relative degree of purity or pollution of water; some aquatic hemipteran are active predators acting as biological control for mosquito larvae [6].

Furthermore, microbial communities play central roles in biogeochemical processes involving abiotic factors such as carbon, nitrogen, and phosphorus cycling [15]. Heavy metals are toxic and harmful, even at low concentrations, to aquatic life and the environment. Especially, metal ions such as copper, silver, and zinc are a serious and ongoing problem. In terms of parameter selection, insects are living indicators for the determination of trace amounts of Ag, Cd, Cu, and Zn in environmental samples [16]. Physical and chemical assessments are inadequate for evaluation and management of fresh water quality. Extremophile organisms are described as organisms that are adapted to grow optimally at or near the extreme ranges of environmental variables [17]. Additionally, bacteria living under extreme conditions were also identified. Hemipterans are likely to be very important ecologically and limnologically, more so than other hexapods [18].

Consequently, unlike our other studies [19, 20], both the intestinal microbiota of water scorpions and some heavy metal accumulations in the insect were studied for the first time in this indicator group. The presence of different bacterial species will accelerate our research. The research area targets wetland sustainability, which is why a wetland where human impact is very high near Erzurum city (Turkey), with a population of approximately half a million people, was chosen. Our results will provide important gains in relation to multiple environmental problems, ecological systems and geographical dimensions.

Material and Methods

Study Area

Adult stage *Nepa rubra* Linnaeus, 1758 were captured using sieves from their natural habitat (Fig. 1) in Erzurum wetland (Erzurum, Turkey). They were brought to the laboratory and maintained at 25ºC in a special container. Two insect samples, male and female, were taken and also repeated once. The study materials (hexapod) were cleaned with a brush before identification and then dissected under a stereo microscope in the laboratory. Morphological identifications [18] were used.

Heavy Metal Analysis

Water scorpion samples were collected during tank experiments with sterile plastic bottles after the regional survey was completed. After water scorpions were sampled, elemental analysis was obtained from Atatürk University East Anatolia High Technology Application and Research Center (DAYTAM) using the 7800 ICP-MS (Inductively Coupled Plasma-

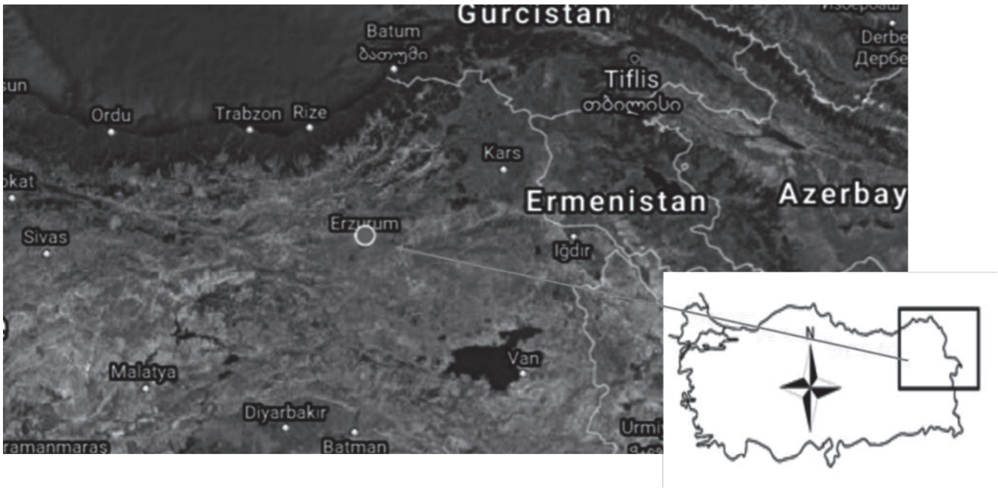


Fig. 1. Study area.

Mass Spectrometer) device. Scientific findings were interpreted [21] and evaluated according to DAYTAM results (Fig. 2).

Analysis Levels of Metagenomics

After removing contaminant microorganisms found on the exoskeleton of the hexapods, surface disinfection was performed with 5 min treatment using 70% alcohol and then washing with sterile distilled water to remove alcohol. The digestive tract of the hexapods had surface disinfection performed and were separated by dissection in a sterile environment. The digestive tract of the hexapod was placed in tubes [19].

After initial denaturation at 95ºC for 7 min, PCR was completed with 35 cycles at 94ºC for 45 s and annealing at 58ºC for 45 s; then extension at 72ºC for 60 s; and a single final polymerization at 72ºC for 5 min before cooling at 15ºC. Library preparation for 16s rRNA V3-V4 amplicon products used Illumina's "Nextera XT DNA Library Prep Kit, Cat. No: FC-131-1096, and the index process was done with "TG Nextera XT Index Kit v2 Set A (96 Indices, 384 Samples), Cat. No: TG-131-2001". The PCR purification processes were done with "AMPure XP beads" from Beckman Coulter Company. Sequencing was done with Illumina's Miseq platform as paired-end (PE) readings of 2x150 base with minimum 30,000 readings per sample. Raw data readings (FASTQ) were QC checked, trimmed (if deemed necessary) and divided into OTU classes with the Kraken Metagenomics system. The Kraken application assigns taxonomic tags to short DNA sequences with high precision and speed [22].

Genomic DNA isolation from bacteria samples were determined fluorometrically. Primer sequences used and PCR conditions are given below; 341F: CCTACGGGNGGCWGCAG 805R: GACTACHVGGGTATCTAATCC.

Following PCR amplification and cloning of the 16S rDNA genes from our isolates, the 16S rDNA gene sequences were determined by using a DNA sequencer sequencing kit (Macrogen, Korea). The sequences consisting of about 1397-1414 nucleotides of the 16S rDNA gene were determined. These sequences were compared with those contained within GenBank by using a BLAST search. Identification and characterization of bacterial strains isolated in this study were performed by using biochemical analysis and genotypic [PCR and 16S rDNA sequence analysis] data. Molecular pairing was performed through

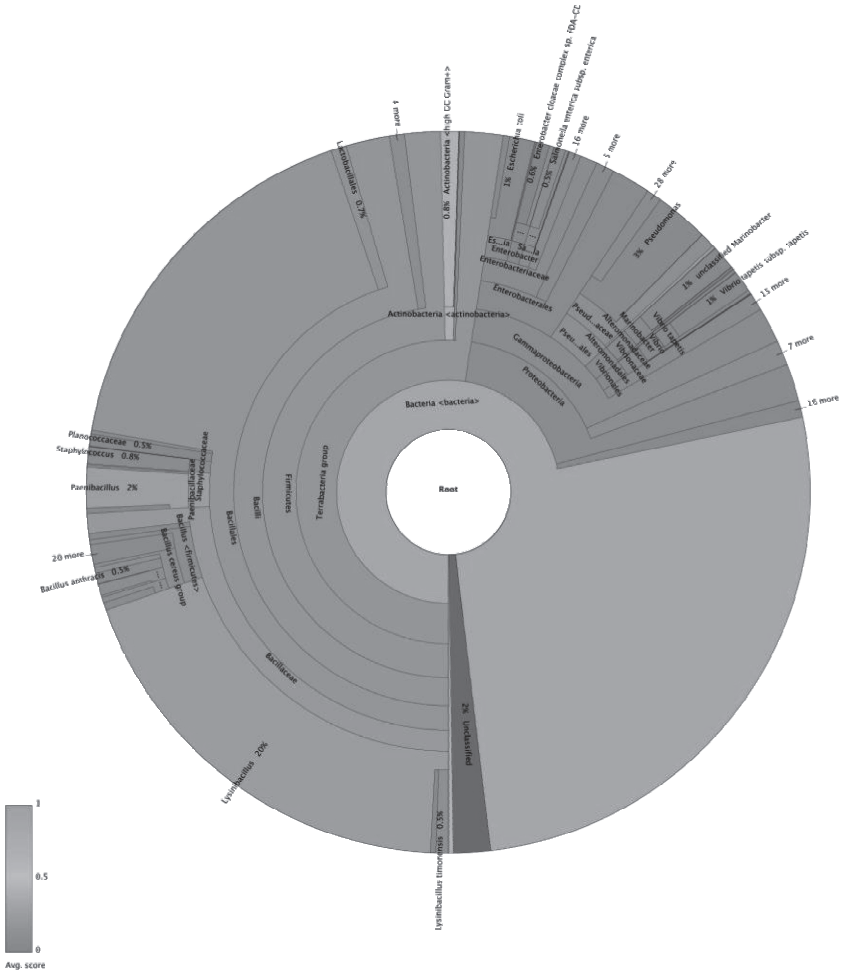


Fig. 2. Bacteria chart.

service procurement from companies performing DNA sequence analysis. The DNA sequences in the strains were then linked to the studies in the National Biotechnology Information Center (NCBI/GenBank). Other applications were obtained via procurement services from a genetic research company [23].

Statistical Evaluation

Bacterial distributions and heavy metal accumulations [21] were evaluated graphically.

Results and Discussion

Hexapods perform important ecosystem services as parasites, predators, biological indicators, and pollinators [19], and have medicinal potential as sources of research for anti-microbial and cancer treatments [24]. Nepidae may be utilized in the biological control of mosquito larvae; hence, researchers are investigating the use of predator Hemiptera for mosquito control [6]. Moreover, this family act as species indicators [25]. Due to having longer respiratory siphons, living samples of *Nepa* spp. [25] were easily identified by siphon length and were selected for research of gut microbiota.

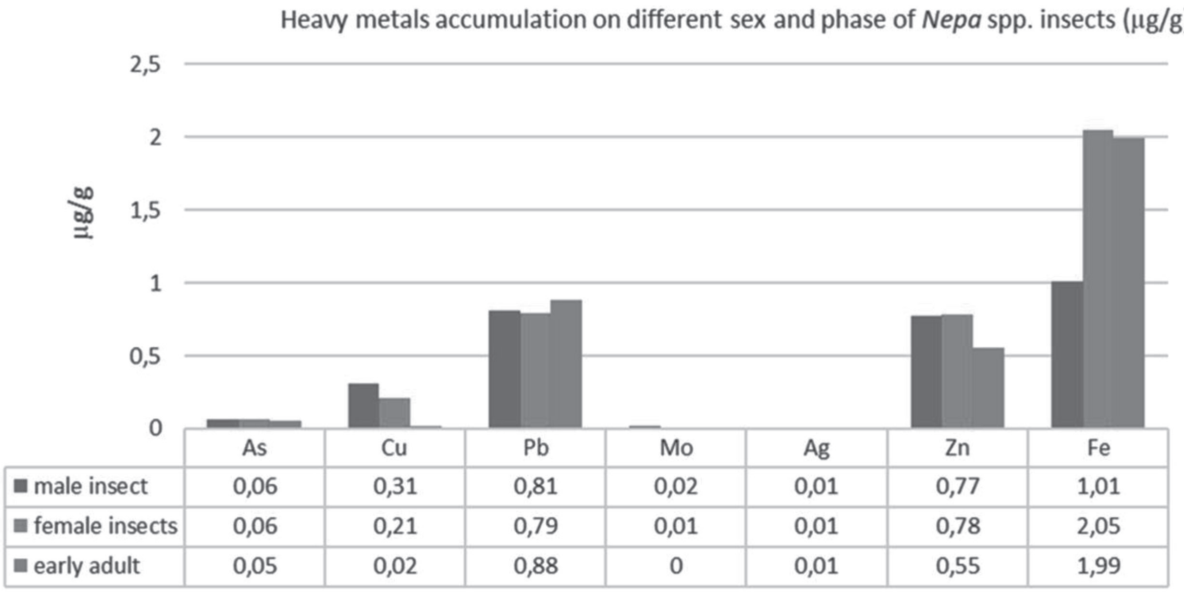
Table 1. Relationships of Endosymbiont and *Canditatus*.

No	Relations species	Primary/Secondary	Canditatus/Endosymbiont
1	*Zixibacteria bacterium*	Primary	candidate division
2	*Annandia adelgestsuga*	Primary	Candidatus
3	*Arsenophonus lipoptenae*	Primary	Candidatus
4	*Baumannia cicadellinicola*	Primary	Candidatus
5	*Carsonella ruddii*	Primary	Candidatus
6	*Chazhemtobacterium aquaticus*	Primary	Candidatus
7	*Cloacimonas acidaminovorans*	Primary	Candidatus
8	*Fukatsuia symbiotica*	Primary	Candidatus
9	*Hamiltonella defensa*	Primary	Candidatus
10	*Liberibacter solanacearum*	Primary	Candidatus
11	*Nanopelagicus hibericus*	Primary	Candidatus
12	*Ruthia magnifica*	Primary	Candidatus
13	*Pseudomonas adelgestsugas*	Primary	Candidatus
14	*Promineofilum breve*	Primary	Candidatus
15	*Phycorickettsia trachydisci*	Primary	Candidatus
16	*Paracaedibacter acanthamoebae*	Primary	Candidatus
17	*Nitrotoga* sp. AM1P 1.0	Primary	Candidatus
18	*Ishikawaella capsulata*	Primary	Candidatus
19	*Sodalis pierantonius*	Primary	Candidatus
20	*Syntrophocurvum alkaliphilum*	Primary	Candidatus
21	*Polyplax serrata*	Primary	Endosymbiont (*Legionella*)
22	*Henestaris halophilus*	Primary	Endosymbiont (*Sodalis)*
23	*Polyrhachis* (*Hedomyrma*) *turneri*	Primary	Endosymbiont (*Blochmannia*)
24	Euscepes postfasciatus	Primary	Endosymbiont
25	*Plateumaris braccata*	Primary	Endosymbiont (Enterobacteriaceae)
26	*Polyplax serrata*	Primary	Endosymbiont (*Legionella*)
27	*Ctenarytaina eucalypti*	Secondary	Endosymbiont
28	*Heteropsylla cubana*	Secondary	Endosymbiont
29	*Henestaris halophilus*	Primary	Endosymbiont (*Sodalis*)

Therefore, the results of metagenomics analysis of gut microbiota of the hexapod are listed on the last page of this article. In addition, the influence of microbial community composition on microbial functional genes remains unclear in the aquatic ecosystem [14] and in relation to insecticide use. The list of bacteria is available at the end of the article.

Wetlands play important roles in global element cycling [26]. Freshwater ecosystems support approximately 10% of all species in the world. Freshwater biodiversity plays an essential role and provides numerous goods and services for the increasing human population [27]. Hemiptera species are less known from research in the Eastern Anatolia region of Turkey [28]. *Nepa cinerea* (*N. rubra*) were reported in Turkey [29]. Nepidae may be utilized in the biological control of mosquito larvae, and researchers are investigating the use of predator hemiptera for mosquito control [6]. As indicator species, *Nepa* spp. [30] and aquatic insects help to indicate the relative degree of purity or pollution of water. Some aquatic hemipteran are active predators acting as biological control of mosquito larvae [6]. Furthermore, *Nepa* spp. adults also spend the winter season under water [31] and are abundantly found in aquatic habitats under leaves, mud and stones. As much as geological location, the diversity of climate and vegetation in Turkey has also enhanced the diversity of insects that belong to the heteropteran order [32]. Therefore, we researched the metagenomics analysis of gut microbiota in a model hexapod because the total potential of microbial communities is very important to understand wetland ecosystem functions. For example, *Bacillus thuringiensis* (Berliner, 1915) is the best-known base for most commercial bio insecticides [11]. *Rhodococcus* sp. and Nocardiaceae are aerobic, nonsporulating, nonmotile gram-positive bacteria [33]; *Azorhizobium caulinodans* participates in nitrogen-fixing symbiosis with plants of the genus *Sesbania* [34] and *Buchnera aphidicola* is an endosymbiont of aphids [35]. Water mite larvae [36], *Barroussia ornata* Schneider 1885, parasitize many aquatic hemiptera; these species infect the gastrointestinal tract of *N. rubra* as host [37]. These bacteria are shown in the list (Table 2). In the bacteria chart (Fig. 3) and the list given in supplementary information, there are several interesting bacteria species were identified in hexapod microbiota. Very important information about endosymbiont relationships were found (Table 1).

Insects respond strongly to heavy metals [30]. These hexapods are possible biomonitor organisms that could be a useful tool for monitoring element contamination [19]. Some symbiosis depends on complementary intracellular solute exchange such as nutrients and carbon compounds [38]. Several non-cultivable bacteria which are endosymbionts that live in animal cells are known [39]. Macroinvertebrate communities broadly reflect environmental conditions and are used as indicators of freshwater quality [40]. For this aim, we selected multivariate methods for the analysis of microbial metagenomics, heavy metals and other components. Cd, Cu, Pb, and Zn, etc. heavy metals are essential for the growth and survival of organisms [41]. However, these metals were widely studied because they cause environmental and public health problems [42]. They are derived from both natural (e.g. rock weathering and soil erosion) and anthropogenic sources. After entering the aquatic ecosystem, only a small portion of free metal ions remains dissolved and the rest are deposited in surface sediments [43]. Typical contamination by these metals (especially Cu, Mn, Cd, Zn and Pb) are toxic threats to the survival and health of organisms in the aquatic ecosystem [44]. Heavy metals pollution is one of the most serious environmental dangers [45] as these abiotic factors are readily absorbed



	As	Cu	Pb	Mo	Ag	Zn	Fe
■ male insect	0,06	0,31	0,81	0,02	0,01	0,77	1,01
■ female insects	0,06	0,21	0,79	0,01	0,01	0,78	2,05
■ early adult	0,05	0,02	0,88	0	0,01	0,55	1,99

Fig. 2. Analysis of heavy metal density.

Table 2. Alphabetic list of bacteria on water scorpion (*Nepa* spp.)' gut microbiota.

A
Abiotrophia defectiva; *Acetilactobacillus jinshanensis*; *Achromobacter insolitus*; *Acidibrevibacterium fodinaquatile*; *Acidothermus cellulolyticus*; *Acidovorax* sp. 16-35-5; *Acinetobacter baumannii*; *Acinetobacter haemolyticus*; *Acinetobacter* sp. WCHAc010034; *Actinobacillus pleuropneumoniae*; *Actinomyces gaoshouyii; Actinotignum schaalii; Aerococcus urinae*; *Aeromonas media*, *A. schubertii*, *A.* sp. 2692-1, *A. veronii*; *Aggregatibacter actinomycetemcomitans*; *Agromyces aureus*; *Alcanivorax* sp. N3-2A; *Alcanivorax xenomutans*; *Alicycliphilus denitrificans*; *Alistipes finegoldii*; *Alkalihalobacillus halodurans*, *A. krulwichiae*; *Alkaliphilus oremlandii*; *Allokutzneria albata*; *Alteromonas macleodii*; *Aminomonas paucivorans*; *Aneurinibacillus soli*, A. sp. XH2; *Anoxybacillus amylolyticus, A. flavithermus; Aquicella lusitana, A. siphonis; Arsenicicoccus sp.* oral taxon 190; *Arsenophonus nasoniae; Arthrobacter citreus*; *Atlantibacter hermannii.*
B
Bacillaceae bacterium; *Bacillus albus, B. altitudinis, B. amyloliquefaciens, B. anthracis, B. cellulosilyticus, B. cereus, B. circulans, B. freudenreichii, B. lentus, B. licheniformis, B. luti, B. megaterium, B. mobilis, B. mycoides, B. pseudomycoides, B. safensis, B. sonorensis, B. subtilis, B. thuringiensis, B. velezensis; Bacterioplanes sanyensis bacterium; Baekduia soli; Basilea psittacipulmonis; Bdellovibrosis; Bacteriovorus; Beijerinckia indica; Bifidobacterium animalis; Blastochloris viridis; Blautia* sp. SC05B48; *Bordetella* sp. H567; *Bos taurus; Bradyrhizobium* sp. SK17; *Brenneria rubrifaciens; Brevibacterium aurantiacum; Brochothrix thermosphacta; Buchnera aphidicola; Burkholderia dolosa, B. oklahomensis, B. thailandensis; Burkholderiales bacterium.*
C
Caldilinea aerophila; *Catenovulum sediminis*; *Cellvibrio* sp. PSBB006; *Chlamydia suis*; *Chloroflexus aggregans*; *Chromatiaceae bacterium* No.7; *Citrobacter amalonaticus*; *Clostridium acetobutylicum, C. beijerinckii, C. bornimense, C. botulinum, C. butyricum, C. estertheticum, C. septicum, C.* sp. CT4, *C.* sp. SYSU GA15002T, *C. taeniosporum, C. tetani*; *Cohnella abietis, C. candidum; Comamonas thiooxydans; Conexibacter woesei; Coraliomargarita akajimensis; Corynebacterium aquilae, C. kroppenstedtii, C. matruchotii, C. segmentosum, C. ureicelerivorans*; *Coxiella burnetii; Cryobacterium arcticum*; *Cupriavidus metallidurans; Cutibacterium acnes; Cyanobacterium aponinum.*
D
Dechloromonas aromatica; Dechloromonas sp. HYN0024; Defluviitoga tunisiensis; Desulfobulbus oralis; Desulfofarcimen acetoxidans; Desulfohalobium retbaense; Desulfomicrobium baculatum; Desulfomonile tiedjei; Desulfosporosinus meridiei; Dichelobacter nodosus; Dickeya chrysanthemi, D. dadantii, D. poaceiphila, D. solani; Dokdonia sp. 4H-3-7-5; *Dolosigranulum pigrum; Dyella thiooxydans.*
E
Ectothiorhodospiraceae bacterium; *Edwardsiella ictaluri; Elusimicrobium minutum*; *E. cloacae* complex sp., *E. cloacae* complex sp. FDA-CDC-AR_0164; *E. hormaechei*, *E. ludwigii*, *E. roggenkampii*; *Enterococcus cecorum*, *E. faecalis*, *E. faecium; Entomoplasma somnilux; Erwinia pyrifoliae*, *E.* sp. Ejp617; *Erysipelothrix larvae*; *Erythrobacter seohaensis*; *Escherichia albertii*, *E. coli*, *E. marmotae; Exiguobacterium* sp. AT1b.
F
Faecalibacterium prausnitzii, F. rodentium; Filifactor alocis; Fimbriimonas ginsengisoli; Francisella marina; Fusobacterium nucleatum.
G
Gallionella capsiferriformans; *Gamma proteobacterium*; *Gemmatimonas phototrophica*; *Geobacillus* sp. GHH01, *G. thermocatenulatus*, *G. thermodenitrificans*, *G. Thermoleovorans*; *Geodermatophilus obscurus*; *Georgenia* sp. Z443; *Geosporobacter ferrireducens*; *Geovibrio thiophilus*; *Gilliamella apicola*; *Gordonibacter urolithinfaciens*.
H
Haliangium ochraceum; *Halobacteriovorax* sp. BALOs; *Halomonas* sp. 1513; *Helicobacter cinaedi*; *Herbaspirillum hiltneri*; *Histophilus somni*; *Hungateiclostridium clariflavum; Hungateiclostridium saccincola*; *Hydrocarboniclastica marina; Hydrogenophaga* sp. PAMC20947; *Hydrogenophilus thermoluteolus; Hyphomicrobium nitrativorans; Hyphomicrobium* sp. MC1.
I
Iamiaceae bacterium; *Ilumatobacter coccineus; Intestinibaculum porci; Izhakiella* sp. KSNA2.
J
Jeotgalibacillus malaysiensis; *Jeotgalicoccus saudimassiliensis*.
K
Kibdelosporangium phytohabitans; *Kiritimatiellaeota bacterium*; *Klebsiella aerogenes*, *K. huaxiensis*, *K. michiganensis*, *K. pneumoniae*, *K. variicola*; *Kluyvera* sp. PO2S7; *Kocuria turfanensis*; *Kosakonia cowanii*, *K. oryzae*; *Ktedonosporobacter rubrisoli*; *Kurthia* sp. 11kri321, *K. zopfii*.

Table 2. Continued.

L
Laceyella sacchari; Lachnoclostridium phytofermentans; Lachnospiraceae bacterium; Lacimicrobium alkaliphilum; Lactiplantibacillus plantarum; Lactobacillus bombi, L. brevis, L. buchneri, L. delbrueckii, L. dextrinicus, L. futsaii, L. jensenii, L. parabuchneri, L. paracasei, L. paracollinoides, L. paraplantarum, L. reuteri, L. rossiae, L. ruminis, L. sanfranciscensis; *Lancefieldella parvula*; *Lawsonella clevelandensis*; *Lawsonia intracellularis*; *Legionella adelaidensis*, *L. clemsonensis*, *L. fallonii*, *L. longbeachae*, *L. pneumophila*, *L. spiritensis*; *Lentibacillus* sp. CBA3610; *Lentzea guizhouensis; Leptospira santarosai*; *Limosilactobacillus fermentum*; *Listeria monocytogenes*; *L.* sp. PSOL-1, *L. weihenstephanensis*; *Litoricola lipolytica; Luteitalea pratensis*; *Luteolibacter* sp. G-1-1-1; *Lysinibacillus* sp. 2017, *L. sphaericus*, *L. timonensis*; *Lysobacter soli*.
M
Magnetospirillum gryphiswaldense; *Malassezia restricta*; *Mannheimia granulomatis*; *Marichromatium purpuratum*; *Marinilactibacillus* sp. 15R; *Marinithermus hydrothermalis*; *Marinobacter hydrocarbonoclasticus*; *M. salarius*; *Marinobacterium* sp. LSUCC0821; *Marinomonas posidonica*; *Meiothermus ruber*; *Mesorhizobium oceanicum*; *Mesorhizobium* sp. DCY119; *Mesorhizobium terrae; Methyloceanibacter* sp. wino2; *Methylomicrobium album*; *Methylomusa anaerophila*; *Microbacterium chocolatum*; *Micropruina glycogenica*; *Mixta theicola*; *Mobiluncus curtisii*; *Moraxella bovoculi*, *M.* osloensis; *Moraxellaceae bacterium*; *Morganella morganii*; *Moritella viscosa*; *Mycoavidus cysteinexigens; Mycobacterium kansasii*, *M. marinum*, *M. tuberculosis*, *M. doricum*, *M. vaccae*; *Mycoplasma capricolum*, *M. mobile*, *M. orale*, *M. penetrans*, *M.* sp. (ex *Biomphalaria glabrata*); *Mycoplasmopsis columbina*.
N
Neisseria animaloris, *N. gonorrhoeae*; *Neisseriaceae bacterium*; *Neochlamydia* sp. S13; *Neorickettsia helminthoeca; Nitrosomonas communis*, *N. europaea*; *Nocardia terpenica; Nocardioides* sp. 603; *Nordella* sp. HKS 07; *Nostoc* sp. TCL240-02; *Novibacillus thermophilus*.
O
Oblitimonas alkaliphila; *Oceanisphaera avium*; *Oleiphilus messinensis*; *Oleispira antarctica*; *Orbus* sp. IPMB12; *Oryzomicrobium terrae*.
P
Paenibacillus alvei, P. barcinonensis, P. bovis, *P. brasilensi, P.cellulosilyticus, P.cellulositrophicus, P. chitinolyticus, P. donghaensis, P. larvae, P. physcomitrellae, P. polymyxa, P. protaetiae, P. thiaminolyticus, P. xylanexedens*, *P. xylanilyticus, P. yonginensis; Paeniclostiridium sordellii; Paenisporosarcina antarctica*; *Pajaroellobacter abortibovis; Pantoea dispersa, P. stewartii; Paraburkholderia rhizoxinica; Parachlamydia acanthamoebae; Paraclostridium bifermentans; Paracoccus kondratievae, P.* sp. AK26, *P.* sp. Arc7-R13, *P. yeei; Paraglaciecola psychrophila; Paraliobacillus* sp. X-1125; *Pasteurella multocida, P. skyensis; Pectobacterium parmentieri; Pediococcus acidilactici, P. damnosus; Pelolinea submarina; Pelosinus fermentans*, *P.* sp. UFO1; *Peptoniphilus ivorii; Peptostreptococcaceae bacterium* oral taxon 929; *Peribacillus muralis; Photobacterium damselae; P. gaetbulicola; Photorhabdus asymbiotica; Phycicoccus* sp. HDW14; *Planctomycetes bacterium*; *Planctomycetes bacterium* Poly30; *Planococcus antarcticus, P.* sp. MB-3u-03; *Plautia stali symbiont; Prevotella denticola; Propionibacterium virus*; *Proteus mirabilis; Providencia sneebia; Pseudarthrobacter phenanthrenivorans; Pseudarthrobacter sulfonivorans; Pseudoclostridium thermosuccinogenes*; *Pseudolabrys* sp. FHR47; *Pseudomonas aeruginosa*, *P. agarici*, *P.* azotoformans, *P. balearica*, *P. entomophila*, *P. fluorescens, P. libanensis, P. litoralis, P. marincola, P. mediterranea, P. mendocina, P. moraviensis, P. oryzae, P. pohangensis, P. putida, P. sabulinigri, P. stutzeri, P. synxantha, P. syringae; Pseudorhodobacter* sp. S12M18; *Pseudothermotoga hypogea; Psychrobacillus glaciei.*
R
Ralstonia mannitolilytica; Ralstonia pickettii; Raoultella ornithinolytica; Reinekea forsetii; Rhizobacter gummiphilus; Rhizobium leguminosarum; Rhodobacter capsulatus, *R. sphaeroides; Rhodococcus erythropolis, Rhodococcus* sp. 008, *R.* sp. SGAir0479; *Rhodoferax ferrireducens; Rhodoplanes* sp. Z2-YC6860*; Rhodothermus marinus; Romboutsia hominis; Romboutsia* sp. CE17; *Roseiflexus castenholzii; Rothia dentocariosa; Rubrobacter xylanophilus; Ruminococcaceae bacterium*; *Ruminococcus* sp. JE7A12; *Ruthenibacterium lactatiformans.*
S
Salinibacter ruber; *Salmonella bongori*, *S. enterica*, *S.* sp. HNK130; *Scandinavium goeteborgense*; *Scytonema* sp. HK-05; *Serratia plymuthica*, *S. rubidaea*, *S.* sp. 3ACOL1, *S. symbiotica*; *Shewanella benthica*, *S. bicestrii*, *S. japonica*; *Shigella dysenteriae*, *S. flexneri*; *Silvanigrella aquatica*; *Simiduia agarivorans*; *Solibacillus silvestris*; *S.* sp. R5-41; *Spiribacter salinus*; *Spiribacter* sp. 2438; *Spirosoma* sp. I-24; *Spongiibacter* sp. IMCC21906; *Sporosarcina pasteurii, S. psychrophila, S. sp. P33; Staphylococcus argenteus, S. aureus, S. auricularis, S. capitis, S. cohnii, S. condimenti, S. epidermidis, S. equorum, S. haemolyticus, S. hominis, S. lugdunensis, S. nepalensis*, *S. pettenkoferi*, *S. pseudintermedius*, *S.* sp. AntiMn-1, *S.* sp. SDB 2975, *S. warneri*; *Stenotrophomonas maltophilia*; *Streptobacillus moniliformis*; *Streptococcus iniae*, *S. mutans*, *S. pneumoniae*, *S. respiraculi*, *S.* sp. Z15, *S. thermophilus*; *Streptomyces parvulus*; *Streptomyces peucetius*; *Streptomyces* sp. 3211, *S.* sp. ICC1; *Sulfurivermis fontis; Synechococcus* sp. JA-3-3Ab; *Syntrophobacter fumaroxidans.*

Table 2. Continued.

T
Tepidiforma bonchosmolovskayae; Terribacillus goriensis; Tessaracoccus timonensis; Thalassolituus oleivorans; Thermobispora bispora; Thermochromatium tepidum; Thermomicrobium roseum; Thermovirga lienii; Thermus oshimai; Thioalkalivibrio sp. K90mix*; Thiobacillus denitrificans; Thioflavicoccus mobilis; Thiomicrorhabdus* sp. aks77*; Thioploca ingrica; Treponema* sp. OMZ 804*; Turicibacter sanguinis, T.* sp. H12.
U
Ureibacillus thermosphaericus.
V
Vagococcus penaei, *V.* sp. MN-17; *Veillonella atypica*, *V. parvula*; *Verrucomicrobium spinosum*; *Vibrio anguillarum*, *V. cholerae*, *V. furnissii*, *V. mimicus, V.* natriegens, *V. parahaemolyticus*, *V. ponticus*, *V. tapetis*; *Virgibacillus* sp. 6R, *V.* sp. Bac330, *V.* sp. SK37.
W
Weissella cryptocerci; *Winogradskyella* sp. HL857; *Wolbachia pipientis*.
X
Xanthobacter autotrophicus; Xanthomonas oryzae; *Xenorhabdus bovienii*, *X. nematophila*; *Xylophilus* sp. KACC 21265.
Y
Yersinia enterocolitica, Y. entomophaga, Y. frederiksenii, Y. pestis.
Z
Zobellella denitrificans.

into tissues of aquatic organisms [46]. In a study about concentrations of heavy metal, copper and cadmium in a biological treatment system, they had negative effects on heterotrophic bacteria concentration [47]. Attempts were made to associate the proportion of heavy metals and traces in water with bacteria in gut microbiota of hexapods. Especially, extremophile and endosymbiont bacteria are firstly discussed in terms of heavy elements. The aim of this study was to examine and ensure verification with metagenomics analysis of gut microbiota in the aquatic hexapod, and to evaluate the correlation between microbial flora and accumulation of heavy metals. Meanwhile, *Arsenicicoccus* sp. bacteria (Table 2) is very remarkable as an environmental factor. Representatives of *Arsenicicoccus* are capable of metabolizing arsenic from wastewater [48] and arsenic is a hazardous heavy metal [49].

Furthermore, we studied the endosymbiont bacteria with their hosts on a molecular basis in gut microbiota of *N. rupra* (Tables 1 and 2). Separately, extremophile organisms principally have salinity tolerance in aquatic environments. Sometimes aquaculture and canal construction have facilitated thousands of alien species becoming established in freshwater [50]. When compared with results from heavy metal and microbiota research in contaminated-polluted areas, our data will be important in the context of bio-index scales.

Conclusions

Accumulation of heavy metal pollutants in living organisms is important to analyze for genomic and microbiota sustainability. Our results highlight the importance of a compartmentalized approach to environmental variables within metacommunity patterns. More investigations on more scales that are dynamic are recommended.

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Conflicts of Interest

The authors declare no conflicts of interest.

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