Communication

Diversity of Bacterial Biota in *Capnodis tenebrionis* (Coleoptera: Buprestidae) Larvae

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Abstract: The bacterial biota in larvae of *Capnodis tenebrionis*, a serious pest of cultivated stone-fruit trees in the West Palearctic, was revealed for the first time using the MiSeq platform. The core bacterial community remained the same in neonates whether upon hatching or grown on peach plants or an artificial diet, suggesting that *C. tenebrionis* larvae acquire much of their bacterial biome from the parent adult. Reads affiliated with class levels *Gammaproteobacteria* and *Alphaproteobacteria* (phylum *Proteobacteria* ca. 86%), and *Actinobacteria* (ca. 14%) were highly abundant. Most diverse reads belong to the families *Xanthomonadaceae* (50%), *Methylbacteriaceae* (20%), *Hyphomicrobiaceae* (9%), *Micrococccaceae* (7%) and *Geodermatophilaceae* (4.5%). About two-thirds of the reads are affiliated with the genera *Lysobacter*, *Microvirga*, *Methylobacterium*, and *Arthrobacter*, which encompass species displaying cellulolytic and lipolytic activities. This study provides a foundation for future studies to elucidate the roles of bacterial biota in *C. tenebrionis*.

Keywords: bacterial biota; Buprestidae; *Capnodis*; stonefruit

1. Introduction

Insect pests may pose significant challenges to environmental quality and human welfare [1], but contribution of their symbiotic microorganisms for establishment and cause additional environmental and economic injury is still vague [2]. Multispecies microbial communities harbored in insect guts are involved in nutrition, digestion, and defense activities. Little is known about the diversity, physiology, and ecology of microorganisms associated with bark and wood-boring beetles [3,4]. Insect-symbiotic bacteria supplement essential nutrients, degrade complex dietary polymers and plant toxins [5–7], and thus contribute to overcoming plant defenses and higher host fitness [8,9]. Flatheaded borers *Capnodis* spp. (Coleoptera: Buprestidae) inflict serious harm to fruit and ornamental trees around the Mediterranean, in Southern Europe and in Western Asia [10]. Control regimes of *Capnodis* spp. populations rely on intensive applications of synthetic insecticides, whereas environmentally friendly means are partially absent. *Capnodis tenebrionis* is the most notorious...
pest among the congeners. Revealing the bacterial biota in larvae may be an avenue to the development of new, safe tools to cope with this pest. Information about microorganisms harbored by C. tenebrionis larvae does not exist; studying their core microbiota can improve the ability to develop more effective management approaches for this pest [11].

2. Results and Discussion

The number of reads per sample that passed a set of sequence filters range from 106,249 to 144,439, with lengths of about 495 bp. The rarefaction analysis allowed for a comparison of species richness (number of OTUs) between different larvae and a determination of adequacy of sequencing output for each feeding regime. All rarefaction curves show the same slope and reach the plateau with 30,000 reads per sample (Figure 1).

![Figure 1. Rarefaction curves represent observed OTUs from C. tenebrionis under different feeding regimes.](image)

The unweighted and weighted UniFrac phylogenetic distance metric plots, obtained using a principal coordinate analysis (PCoA), allowed for an assessment of microbial community differences between larvae of C. tenebrionis, neonates, and those grown on either peach plants or an artificial diet (Figure 2). The unweighted UniFrac distances (Figure 2A) show that compositions of these samples were identical, but the weighted distances (Figure 2B) display a variance in the communities’ composition that is unexplained by diet type. Thus, diet type seems not to affect the bacterial biota composition of C. tenebrionis larvae. In other species, larval diet does affect the microbiota composition [6,12]. The pine weevil beetle Hylobius abietis is, however, also resilient to changes in diet [9]: their microbiome is the same whether they were fed on an artificial diet or on Norway spruce twigs.
Most abundant reads in all *C. tenebrionis* larvae were affiliated with phyla of *Proteobacteria* (84–87%) and *Actinobacteria* (12–15%) (Figure 3; Table 1). At the class level, they were assigned to *Gammaproteobacteria* (46–52%), *Alphaproteobacteria* (32–39%), and *Actinobacteria* (12–15%) (Figure 3; Table 2).

### Table 1. Taxonomy assignment at the phylum level of *C. tenebrionis* on different feeding regimes.

| Phylum         | Artificial#1 | Artificial#2 | Neonates#1 | Neonates#2 | Peach#1 | Peach#2 |
|----------------|--------------|--------------|------------|------------|---------|---------|
| *Proteobacteria* | 87.22%       | 85.12%       | 86.60%     | 85.21%     | 83.86%  | 84.69%  |
| *Actinobacteria* | 12.09%       | 14.25%       | 12.83%     | 14.23%     | 15.38%  | 14.85%  |
| Unassigned     | 0.54%        | 0.56%        | 0.49%      | 0.37%      | 0.42%   | 0.35%   |
| Others         | 0.15%        | 0.07%        | 0.08%      | 0.18%      | 0.34%   | 0.11%   |

1 Others = Bacteroidetes, Chloroflexi, Firmicutes, Planctomycetes, Cyanobacteria.

### Table 2. Taxonomy assignment at the class level of *C. tenebrionis* on different feeding regimes.

| Class              | Artificial#1 | Artificial#2 | Neonates#1 | Neonates#2 | Peach#1 | Peach#2 |
|--------------------|--------------|--------------|------------|------------|---------|---------|
| *Gammaproteobacteria* | 47.69%       | 50.47%       | 46.49%     | 51.49%     | 49.67%  | 52.31%  |
| *Alphaproteobacteria* | 39.13%       | 33.80%       | 39.65%     | 33.28%     | 33.79%  | 31.88%  |
| *Actinobacteria*   | 12.07%       | 14.23%       | 12.79%     | 14.21%     | 15.35%  | 14.83%  |
| Unassigned         | 0.54%        | 0.56%        | 0.49%      | 0.37%      | 0.42%   | 0.35%   |
| Others             | 0.58%        | 0.94%        | 0.57%      | 0.64%      | 0.77%   | 0.63%   |

1 Others = Betaproteobacteria, Cytophagia, Acidimicrobia, Thermomicrobia, Bacilli, Phycisphaerae, Chloroplast, Flavobacteria.
Figure 3. Taxonomy assignment at the family level of *C. tenebrionis* on three different feeding regimes.

| Feeding Regime | Taxonomy Assignment |
|----------------|---------------------|
| Artificial 1 | Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae |
| Artificial 2 | Proteobacteria; Alphaproteobacteria; Rhizobiales; Methyllobacteriaceae |
| Peach 1      | Proteobacteria; Alphaproteobacteria; Rhizobiales; Hyphomicrobiaceae |
| Peach 2      | Actinobacteria; Actinobacteria; Micrococcaceae; Micrococcales |
| Neonates 1   | Actinobacteria; Actinobacteria; Frankiales; Geodermatophilaceae |
| Neonates 2   | Proteobacteria; Alphaproteobacteria; Rhizobiales; Xanthobacteraceae |
|              | Actinobacteria; Actinobacteria; Propionibacteriales; Propionibacteriaceae |
|              | Unassigned |
| Artificial 2 | Actinobacteria; Actinobacteria; Actinobacteria; Other |
| Peach 1      | Proteobacteria; Alphaproteobacteria; Rhizobiales; Other |
| Peach 2      | Proteobacteria; Betaproteobacteria; Burkholderiales; Oxalobacteraceae |
| Neonates 1   | Proteobacteria; Other |
| Neonates 2   | Proteobacteria; Alphaproteobacteria; Rhizobiales; JG34-KF-361 |
|              | Actinobacteria; Actinobacteria; Propionibacteriales; Propionibacteriaceae |
|              | Bacteroidetes; Cytophagia; Cytophagales; Cytophagaceae |
|              | Proteobacteria; Alphaproteobacteria; Rhodospirillales; Acetobacteraceae |
|              | Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae |
|              | Actinobacteria; Acidimicrobiia; Acidimicrobiaceae |
|              | Chloroflexi; Thermomicrobia; JG30-KF-CM45; uncultured bacterium |
|              | Firmicutes; Bacilli; Bacillales; Paenibacillaceae |
|              | Planctomycetes; Phycisphaerae; Tepidisphaerales; Tepidisphaeraceae |
|              | Bacteria; Other |
| Artificial 2 | Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae |
|              | Cyanobacteria; Chloroplast; uncultured bacterium; |
| Peach 1      | Proteobacteria; Alphaproteobacteria; Sphingomonadales; Erythrobacteraceae |
| Peach 2      | Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae |
| Neonates 1   | Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae |
| Neonates 2   | Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae |

**Figure 3.** Taxonomy assignment at the family level of *C. tenebrionis* on three different feeding regimes.
The most abundant (ca. 50%) reads were affiliated with the Xanthomonadaceae family within the Gammaproteobacteria class (Figure 3), where 45% of them were not identified at the genus level using the Silva database as a reference. Blastn analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi), however, revealed that these reads are affiliated with genus Lysobacter, members of which are Gram negatives widely distributed in soil, plant, and freshwater habitats. Reads affiliated with Lysobacter were one of the most prevalent groups in the gut flora of herbivorous cephalotine ants (Cephalotes varians, C. rohweri, and C. atratus) [13]. Cellulase and glucanase activities [14] were identified in some Lysobacter species: IB-9374 [15], L. capsici AZ78 [16], and L. enzymogenes strain N4-7 [17]. Moreover, L. enzymogenes controls phytopathogenic nematodes [18] and fungi such as Bipolaris sorokiniana [19], Uromyces appendiculatus [20], Fusarium graminearum [21], and Rhizoctonia solani [22]. Lysobacter gumnosus that lives on redback salamanders’ skin produces 2,4-diacetylphloroglucinol, which inhibits the growth of certain pathogenic fungi [23].

The second most abundant (ca. 20%) reads are affiliated with the Methylobacteriaceae family (order Rhizobiales, class Alphaproteobacteria) (Figure 3). The blastn revealed that 91% (ca. 65%, similarity cut-off 96%; 26%, similarity cut-off 97%) of the all Methylobacteriaceae are affiliated with genus Microvirga, 7.5% are classified as genus Methylobacterium, and the rest (about 1.5% reads) were identified as uncultured Methylobacteriaceae.

The Microvirga (formerly Balneimonas) and Methylobacterium species are ubiquitous in nature, mainly soil and water, but also plants’ phylloplane [24–26]. Bacteria of the Microvirga and Methylobacterium genera have recently been found essential for efficient digestion of lignocellulose in the gut of the wood-feeding termite Reticulitermes chinensis (Isoptera: Rhinotermitidae) [27], that digest glucosyl and xylosyl residues from lignocellulose [28]. Methylobacterium spp. perform lipolytic activity in weevils (Coleoptera: Curculionidae) and may play a role in nutritional processes [29]. The abundance (ca. 20%) of Microvirga and Methylobacterium in C. tenebrionis may therefore be involved in cellulolytic and lipolytic activities.

The third most abundant (ca. 9%) reads are affiliated with the Hyphomicrobiaceae family within the Alphaproteobacteria class (Figure 3). Analysis by blastn classified them to the genus Devosia (similarity cut-off 96%). Cellulolytic bacteria belonging to this genus were isolated from the larval gut of root-feeding Holotrichia parallela (Coleoptera: Scarabaeidae) [30] and of silkworm [31]. It is reasonable that the flat-headed root borer C. tenebrionis harbors these cellulolytic bacteria.

The fourth most abundant (7%) reads are affiliated with the Micrococcaceae family (class Actinobacteria) (Figure 3). Analysis by blastn affiliated most of them with the Gram-positive coryneform genus Arthrobacter (similarity cut-off 97%), members of which are commonly found in soil. Arthrobacter gandensis and A. gandavensis were isolated from the wireworm Agriotes lineatus (L.) (Coleoptera: Elateridae), a serious pest of various vegetables and fruits throughout the world [32]. Arthrobacter pityocampa was isolated from the pine processionary moth Thaumetopoea pityocampa (Lepidoptera: Thaumetopoidae), one of the most harmful pests of pine species in Mediterranean countries [33]. Arthrobacter spp. are prevalent in the gut microbiota of the cave beetles Neobathyscia pasai and N. mancini (Coleoptera: Leiodidae) [34]. Wood-digesting Arthrobacter sp., which had been isolated from the hind-gut of the termite Reticulitermes hesperus [35], was also found in the microbiome of C. tenebrionis.

The fifth most abundant (4.5%) reads are affiliated with the Geodermatophilaceae family (order Actinomycetales) (Figure 3). Analysis by blastn affiliated most of them with the genus Blastococcus (similarity cut-off 97%). Members of the family Geodermatophilaceae contain bacteria isolated mainly from soils, seawater, and stone surfaces [36]. Little is known about Blastococcus, but reads belonging to this genus have been retrieved from globally important pest, the chilli thrips Scirtothrips dorsalis (Thysanoptera: Thripidae) [37] and strains affiliated with Geodermatophilus, were found associated with Paratrechina longicornis (Hymenoptera: Formicidae) [38].

Apart from habitat- and diet-specific microbes, an insect’s gut harbors a core microbiome, members of which have likely co-evolved with the host and fulfill important functions, such as...
cellulose degradation [39], breakdown of ingested toxins, or overcome chemicals used for insect control [6,40,41]. Indigenous bacteria are often specialized gut symbionts and are transmitted vertically from the eggs, through coprophagy or social interactions. Gut communities of social insects are usually more distinctive and consistent than those of non-social invertebrates [6]. The findings described here reveal a high similarity of microbial communities retrieved from C. tenebrionis neonates (hatching larvae before any act of feeding) or reared on either peach plants or an artificial diet. These findings imply that the larvae acquire much of their bacterial biome from the parent adult.

Gut symbionts may have the potential to protect their host from insecticides such as fenitrothion [40]. In gypsy moth larvae, on the other hand, elimination of the indigenous midgut microbial community abolished insecticidal activity of Cry’s, and re-introduction of a specific member of this community restored Bacillus thuringiensis-mediated killing [42]. Bacterial symbionts may be utilized to manage insect pests [6] in different ways: insecticidal potential of entomopathogenic gut bacteria may serve for pest management; genetically modified bacteria can be used as vehicles to specifically express foreign traits that interfere fitness of the pest; gut symbiont that naturally inhibit parasite colonization could be disseminated in insect populations, for example, to prevent spread of human disease via insect vectors or influence vector competence by modulating immune responses. Characterizing the core microbiota of C. tenebrionis larvae is essential for understanding their physiology and ecology, and thus could be helpful in developing the next generation of pest control strategies.

3. Materials and Methods

Adults of C. tenebrionis were collected in nectarine orchard next to Yesod HaMa’ala (Hulla Valley, 33°05’26” N, 35°58’90” E, and newly-hatched larvae of the first lab generation were examined. Larvae were randomly sampled from hatching neonates, and from individuals lab-reared on peach plants and on an artificial diet over four weeks [43]. The larvae were surface-sterilized with 75% ethanol for 90 s, rinsed twice with sterilized-deionized water, and stored at −80 °C.

Total bacterial DNA was extracted from the larvae using the PowerSoil® DNA Isolation Kit (MO BIO laboratories, San Diego, CA, USA) following the manufacturer’s protocol, except that each sample was placed into liquid nitrogen and crushed with a pestle before cell lysis. Total genomic DNA extraction of 6 samples, each composed of 3 C. tenebrionis larvae, was performed by the DNA Services Facility at the Research Resources Center, at the University of Illinois at Chicago, for 16S rRNA gene sequencing using the Illumina MiSeq platform. Conserved regions V1–V3 of the 16S rDNA were amplified using PCR with the pair of primers CS1_27F/CS2_534R. A total of 1,056,756 reads were obtained.

Raw reads were merged using the software package PEAR (v0.9.10) [44]. Low quality sequences and chimeras were removed by the software package Mothur (v1.36.1) [45]. The quality-controlled sequences were processed with the Quantitative Insights into Microbial Ecology (QIIME v1.9.1) package [46]. Briefly, sequence data were clustered into operational taxonomic units (OTU) at 97% similarity using UCLUST. Representative sequences from each OTU were extracted and aligned using PyNAST with a percent identity threshold of 60% to the Silva 16S rRNA bacterial database [47]. Representative sequences were classified for taxonomy assignment by UCLUST and Silva database. Unassigned OTUs at the genus level were analyzed using standard nucleotide BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi). A Biological Observation Matrix (BIOM) was generated at different taxonomic levels. OTU with a total observation count lower than 50 reads were discarded. Qiime was also used to generate an alpha rarefaction plot as well as principal coordinate analysis (PCoA) plots based on weighted and unweighted UniFrac metrics (beta diversity).

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References

1. Chornesky, E.A.; Bartuska, A.M.; Aplet, G.H.; Britton, K.O.; Cummings-Carlson, J.; Davis, F.W.; Eskow, J.; Gordon, D.R.; Gottschalk, K.W.; Haack, R.A.; et al. Science priorities for reducing the threat of invasive species to sustainable forestry. *AIBS Bull.* 2005, 55, 335–348. [CrossRef]

2. Vasanthakumar, A.; Handelsman, J.O.; Schloss, P.D.; Bauer, L.S.; Raffa, K.F. Gut microbiota of an invasive subcortical beetle, *Agrilus planipennis* Fairmaire, across various life stages. *Environ. Entomol.* 2008, 37, 1344–1353. [CrossRef] [PubMed]

3. Schloss, P.D.; Delalibera, I., Jr.; Handelsman, J.O.; Raffa, K.F. Bacteria associated with the guts of two wood boring beetles: *Anoplophora glabripennis* and *Saperda vestita* (Cerambycidae). *Environ. Entomol.* 2006, 35, 625–629. [CrossRef]

4. Vasanthakumar, A; Delalibera, I, Jr.; Handelsman, J.; Klepzig, K.D.; Schloss, P.D.; Raffa, K.F. Characterization of gut-associated bacteria in larvae and adults of the southern pine beetle, *Dendroctonus frontalis* Zimmermann. *Environ. Entomol.* 2006, 35, 1710–1717. [CrossRef]

5. Douglas, A.E. The microbial dimension in insect nutritional ecology. *Funct. Ecol.* 2009, 23, 38–47. [CrossRef]

6. Engel, P.; Moran, N.A. The gut microbiota of insects—diversity in structure and function. *FEMS Microbiol. Rev.* 2016, 37, 699–735. [CrossRef]

7. Wéle, C.U.; de Graaf, R.M.; van den Bosch, T.J.; Op den Camp, H.J.; van Dam, N.M.; Jetten, M.S. Plasmids from the gut microbiome of cabbage root fly larvae encode SaxA that catalyzes the conversion of the plant toxin 2-phenylethyl isothiocyanate. *Environ. Microbiol.* 2016, 18, 1379–1390. [CrossRef]

8. Hammer, T.J.; Bowers, M.D. Gut microbes may facilitate insect herbivory of chemically defended plants. *Oecologia* 2015, 179, 1–14. [CrossRef]

9. Berasategui, A.; Salem, H.; Paetz, C.; Santoro, M.; Gershenzon, J.; Kaltenpoth, M.; Schmidt, A. Gut microbiota of the pine weevil degrades conifer diterpenes and increases insect fitness. *J. Nematol.* 2000, 32, 233–239. [CrossRef]

10. Ben-Yehuda, S.; Assael, F.; Mendel, Z. Improved chemical control of *Capnodis tenebrionis* L. and *C. carbonaria* Klug (Coleoptera: Buprestidae) in stone-fruit plantations in Israel. *Phytoparasitica* 2000, 28, 27–41. [CrossRef]

11. Gindin, G.; Mendel, Z.; Levi, T.; Shahi, P.; Moshkapov, M.; Khasdan, V.; Weinthal, D.; Kuznetsova, T.; Einav, M.; et al. The basis for rootstock resilient to *Capnodis* species: Screening for genes encoding δ-endotoxins from *Bacillus thuringiensis*. *Pest Manag. Sci.* 2014, 70, 1283–1290. [CrossRef] [PubMed]

12. Strano, C.P.; Malacrinò, A.; Campolo, O.; Palmeri, V. Influence of host plant on *Thaumetopoea pityocampa* gut bacterial community. *Microb. Ecol.* 2018, 75, 487–494. [CrossRef] [PubMed]

13. Anderson, K.E.; Russell, J.A.; Moreau, C.S.; Kautz, S.; Sullam, K.E.; Hu, Y.I.; Basinger, U.; Mott, B.M.; Duck, N.; Wheeler, D.E. Highly similar microbial communities are shared among related and trophically similar ant species. *Environ. Entomol.* 2012, 41, 2282–2296. [CrossRef] [PubMed]

14. Gómez Exposito, R.; Postma, J.; Raaijmakers, J.M.; De Bruijn, I. Diversity and activity of *Lysobacter* species from disease suppressive soils. *Front. Microbiol.* 2015, 6, 1243. [CrossRef]

15. Ogura, J.; Toyoda, A.; Kurosawa, T.; Chong, A.L.; Chohnan, S.; Masaki, T. Purification, activity, and gene analysis of cellulase (Cel8A) from *Lysobacter enzymogenes* strain C3 on nematodes. *J. Nematol.* 2006, 38, 233–239.
19. Zhang, Z.; Yuen, G.Y. Biological control of Bipolaris sorokiniana on tall fescue by Sterotrophomonas maltophilia strain C3. Phytopathology 1999, 89, 817–822. [CrossRef]
20. Yuen, G.Y.; Steadman, J.R.; Lindgren, D.T.; Schaff, D.; Jochum, C. Bean rust biological control using bacterial agents. Crop Prot. 2001, 20, 395–402. [CrossRef]
21. Jochum, C.C.; Osborne, L.E.; Yuen, G.Y. Fusarium head blight biological control with Lysobacter enzymogenes. Biol. Control 2006, 39, 336–344. [CrossRef]
22. Postma, J.; Schilder, M.T.; Bloem, J.; Van Leeuwen-Haagsma, W.K. Soil suppressiveness and functional diversity of the soil microflora in organic farming systems. Soil Biol. Biochem. 2008, 40, 2394–2406. [CrossRef]
23. Brucker, R.M.; Baylor, C.M.; Walters, R.L.; Lauer, A.; Harris, R.N.; Minbiole, K.P. The identification of 2,4-diacetylphloroglucinol as an antifungal metabolite produced by cutaneous bacteria of the salamander Plethodon cinereus. J. Chem. Ecol. 2008, 34, 39–43. [CrossRef] [PubMed]
24. Kelly, D.P.; McDonald, I.R.; Wood, A.P. The family Methylobacteriaceae. In The Prokaryotes; Springer: Berlin/Heidelberg, Germany, 2014; pp. 313–340.
25. Liu, Y.H.; Guo, J.W.; Salam, N.; Li, L.; Zhang, Y.G.; Han, J.; Mohamad, O.A.; Li, W.J. Culturable endophytic bacteria affiliated with the genera Microvirga, Phyllobacterium, and Bradyrhizobium nodulate Lupinus micranthus growing in soils of Northern Tunisia. Appl. Environ. Microbiol. 2017, 83. [CrossRef]
26. Msaddak, A.; Duran, D.; Rejili, M.; Mars, M.; Argueso, T.R.; Imperial, J.; Palacios, J.; Rey, L. Diverse bacteria of the cave beetles Neobathyscia pasai (Leiodidae; Bignell, D.E., Roisin, Y., Lo, N., Eds.; Springer: Dordrecht, The Netherlands, 2011; pp. 375–412.
27. Zhou, N.; Sun, Y.T.; Chen, D.W.; Du, W.; Yang, H.; Liu, S.J. Harnessing microfluidic streak plate technique to investigate the gut microbiome of Reticulitermes chinensis. MicrobiologyOpen 2018, e00654. [CrossRef]
28. Bignell, D.E. Morphology, physiology, biochemistry and functional design of the termite gut: An evolutionary wonderland. In Biology of Termites: A Modern Synthesis; DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F., Eds.; Springer: Berlin/Heidelberg, Germany, 2014; pp. 313–340.
29. Huang, S.; Sheng, P.; Zhang, H. Isolation and identification of cellulolytic bacteria from the gut of Holotrichia parallela larvae (Coleoptera: Scarabaeidae). Int. J. Mol. Sci. 2012, 13, 2563–2577. [CrossRef]
30. Liang, X.; Fu, Y.; Tong, L.; Liu, H. Microbial shifts of the silkworm larval gut in response to lettuce leaf feeding. Appl. Microbiol. Biotechnol. 2014, 98, 3769–3776. [CrossRef]
31. Danismazoglu, M.; Demir, I.; Sevim, A.; Demirbag, Z.; Nalcacioglu, R. An investigation on the bacterial flora of Agriotes lineatus (Coleoptera: Elateridae) and pathogenicity of the flora members. Crop Prot. 2012, 40, 1–7. [CrossRef]
32. Ince, I.A.; Demirbag, Z.; Kati, H. Arthrobacter pityocampae sp. nov., isolated from Thaumetopoea pityocampa (Lep., Thaumetopoeidae). Int. J. Syst. Evol. Microbiol. 2014, 64, 3384–3389. [CrossRef]
33. Latella, L.; Castioni, A.; Bignott, L.; Savetti, E.; Torriani, S.; Felis, G.E. Exploring gut microbiota composition of the cave beetles Neobathyscia parasai Ruffo, 1950 and Neobathyscia mancinii Jeannel, 1924 (Leiodidae; Cholevinae). Boll. Mus. Civ. Stor. Nat. Verona 2017, 41, 3–24. [CrossRef]
34. Thayer, D.W. Facultative wood-digesting bacteria from the hind-gut of the termite Reticulitermes hesperus. J. Gen. Microbiol. 1976, 95, 287–296. [CrossRef] [PubMed]
35. Normand, P.; Daffonchio, D.; Giori, M. The Family Geodermatophilaceae. In The Prokaryotes; Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F., Eds.; Springer: Berlin/Heidelberg, Germany, 2014.
36. Dickey, A.M.; Tease, A.J.; Jara-Cavieres, A.; Kumar, V.; Christenson, M.K.; Potluri, L.P.; Morgan, J.K.; Shatters, R.G., Jr.; Mckenzie, C.L.; Davis, P.H.; et al. Estimating bacterial diversity in Scirtothrips dorsalis (Thysanoptera: Thripidae) via next generation sequencing. Fla. Entomol. 2014, 97, 362–366. [CrossRef] [PubMed]
37. Reyes, R.D.; Cafaro, M.J. Paratricha longicornis ants in a tropical dry forest harbor specific Actinobacteria diversity. J. Basic Microbiol. 2015, 55, 11–21. [CrossRef] [PubMed]
38. Warnecke, F.; Luginbühl, P.; Ivanova, N.; Ghassemian, M.; Richardson, T.H.; Stege, J.T.; Cayouette, M.; McHardy, A.C.; Djordjevic, G.; Aboushadi, N.; et al. Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. Nature 2007, 450, 560–565. [CrossRef]
40. Kikuchi, Y.; Hayatsu, M.; Hosokawa, T.; Nagayama, A.; Tago, K.; Fukatsu, T. Symbiont-mediated insecticide resistance. *Proc. Natl. Acad. Sci. USA* 2012, 109, 8618–8622. [CrossRef] [PubMed]

41. Ping, L.; Büchler, R.; Mithöfer, A.; Svatoš, A.; Spiteller, D.; Dettner, K.; Gmeiner, S.; Piel, J.; Schlott, B.; Boland, W. A novel Dps-type protein from insect gut bacteria catalyses hydrolysis and synthesis of N-acyl amino acids. *Environ. Microbiol.* 2007, 9, 1572–1583. [CrossRef] [PubMed]

42. Broderick, N.A.; Robinson, C.J.; McMahon, M.D.; Holt, J.; Handelsman, J.; Raffa, K.F. Contributions of gut bacteria to *Bacillus thuringiensis*-induced mortality vary across a range of Lepidoptera. *BMC Biol.* 2009, 7, 11. [CrossRef]

43. Gindin, G.; Kuznetsowa, T.; Protasov, A.; Ben-Yehuda, S.; Mendel, Z. Artificial diet for two flat headed borers, *Capnodis* spp. (Coleoptera: Buprestidae). *Eur. J. Entomol.* 2009, 106, 573–581. [CrossRef]

44. Zhang, J.; Kobert, K.; Flouri, T.; Stamatakis, A. PEAR: A fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* 2014, 30, 614–620. [CrossRef] [PubMed]

45. Schloss, P.D.; Westcott, S.L.; Ryabin, T.; Hall, J.R.; Hartmann, M.; Hollister, E.B.; Lesniewski, R.A.; Oakley, B.B.; Parks, D.H.; Robinson, C.J.; et al. Introducing mothur: Open-source, platform-independent, community supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 2009, 75, 7537–7541. [CrossRef] [PubMed]

46. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Pena, A.G.; Goodrich, J.K.; Gordon, J.I.; et al. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 2010, 7, 335–336. [CrossRef] [PubMed]

47. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Pflieger, J.; Glöckner, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* 2013, 41, D590–D596. [CrossRef] [PubMed]

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