

## Biodegradation of Polystyrene by Dark (*Tenebrio obscurus*) and Yellow (*Tenebrio molitor*) Mealworms (Coleoptera: Tenebrionidae)

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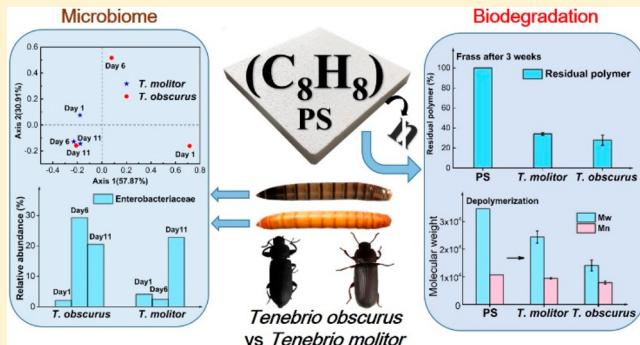
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### S Supporting Information

**ABSTRACT:** Yellow mealworms (larvae of *Tenebrio molitor*, Coleoptera: Tenebrionidae) have been proven to be capable of biodegrading polystyrene (PS) products. Using four geographic sources, we found that dark mealworms (larvae of *Tenebrio obscurus*) ate PS as well. We subsequently tested *T. obscurus* from Shandong, China for PS degradation capability. Our results demonstrated the ability for PS degradation within the gut of *T. obscurus* at greater rates than *T. molitor*. With expanded PS foam as the sole diet, the specific PS consumption rates for *T. obscurus* and *T. molitor* at similar sizes (2.0 cm, 62–64 mg per larva) were  $32.44 \pm 0.51$  and  $24.30 \pm 1.34$  mg 100 larvae<sup>-1</sup> d<sup>-1</sup>, respectively. After 31 days, the molecular weight ( $M_n$ ) of residual PS in frass (excrement) of *T. obscurus* decreased by 26.03%, remarkably higher than that of *T. molitor* (11.67%). Fourier transform infrared spectroscopy (FTIR) indicated formation of functional groups of intermediates and chemical modification. Thermo gravimetric analysis (TGA) suggested that *T. obscurus* larvae degraded PS effectively based on the proportion of PS residue. Co-fed corn flour to *T. obscurus* and wheat bran to *T. molitor* increased total PS consumption by 11.6% and 15.2%, respectively. Antibiotic gentamicin almost completely inhibited PS depolymerization. High-throughput sequencing revealed significant shifts in the gut microbial community in both *Tenebrio* species that were associated with the PS diet and PS biodegradation, with changes in three predominant families (Enterobacteriaceae, Spiroplasmataceae, and Enterococcaceae). The results indicate that PS biodegradability may be ubiquitous within the *Tenebrio* genus which could provide a bioresource for plastic waste biodegradation.



## INTRODUCTION

Plastic wastes have become a global environmental concern, with over 299 million tons of petroleum-based synthetic plastics industrially produced worldwide every year.<sup>1–7</sup> Recently, microplastic particles (i.e., particle diameter <5 mm) have become an emerging environmental concern, as they have been found contaminating rivers, lakes, oceans, and wastewater treatment plants.<sup>8–13</sup> Polystyrene (PS),  $[-\text{CH}-(\text{C}_6\text{H}_5)\text{CH}_2-]_n$ , is one of the major sources of plastic products in the world, with an annual production rate exceeding 20 million tons per year.<sup>14</sup> Due to their high durability, plasticity, and corrosion resistance, PS products are widely used in packing, building, and food processing industries in forms of expanded PS (EPS), trade name Styrofoam, and as extruded PS (XPS). Due to its high prevalence in daily life, PS has

become a major pollutant of soils, rivers, lakes, and oceans and is a source of microplastics.<sup>15–17</sup>

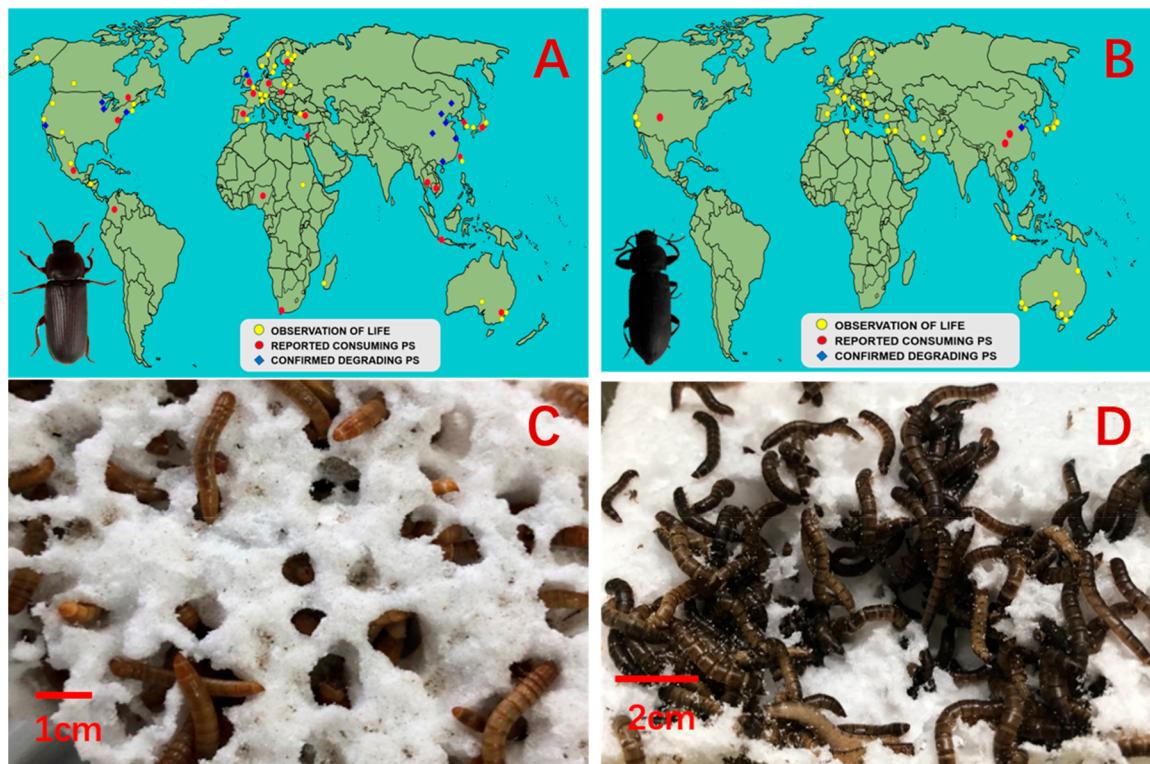
Biodegradation of plastic wastes, including that of PS, has been studied since the 1960s.<sup>18–23</sup> Researchers have attempted to use mixed microbial cultures and isolated bacteria from various sources such as soil, garbage, or sewage sludge to biodegrade PS into low molecular organics or mineralize to CO<sub>2</sub>.<sup>24–28</sup> However, the efficiency of PS biodegradation by microorganisms or enzymes varied because of the persistent macromolecular structure of the plastics and was still quite low

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**Figure 1.** *Tenebrio molitor* and *Tenebrio obscurus* around the world and PS foam-eating behaviors. (a) Discover life and original sources of *T. molitor*. (b) Discover life and original sources of *T. obscurus*. (c) PS foam-eating *T. molitor* larvae and (d) *T. obscurus* larvae from Shandong Province, China. Yellow dots: life and sources of *Tenebrio*; Red dots: larvae eating PS; Blue dots: larvae degrading PS. Data adapted from references<sup>33,41,56,57</sup> and this study.

even with preconditioning or pretreatment processes to the polymer.<sup>28–31</sup>

Recently, PS has been shown to be susceptible to rapid biodegradation and mineralization in the gut of *Tenebrio molitor*.<sup>1,32–35</sup> The larvae of *Tenebrio molitor* Linnaeus 1758, commonly referred to as yellow mealworms, are Coleoptera (beetles) within the cosmopolitan family Tenebrionidae (common name “darkling beetle”), which comprises more than 20000 species. They have been found around the world (Figure 1a) and are capable of ingesting and biodegrading different PS foam products.<sup>1,34,35</sup> A recent study indicated that *T. molitor* populations from 22 countries from Asia, North America, Europe, Africa, and Australia were able to consume PS foam and have an intrinsic capacity for biodegradation of PS, through eating as well as chewing behaviors and through gut microbe dependent oxidative digestive machinery. Brandon et al. (2018) further investigated the biodegradation of mixed plastics [Polyethylene (PE) and PS] through next-generation sequencing and revealed that two OTUs (*Citrobacter* sp. and *Kosakonia* sp.) were strongly associated with both PE and PS biodegradation.<sup>35</sup> In previous research, we hypothesized that the ability of insects to depolymerize diverse plastics is likely ubiquitous and not restricted to *T. molitor*.<sup>33</sup> According to our observations and literature, dark mealworms, larvae of *T. obscurus* (Fabricius 1792), superworms (larvae of *Zophobas morio*; Coleoptera: Tenebrionidae) (Figure S1a), and other beetles (*Trogoderma variabile*, *Lasioderma serricorne*, *Rhyzopertha dominica*, *Tenebrioides mauretanicus*, among others in Order Coleoptera) can chew, eat, and penetrate various plastic packing materials.<sup>36–38</sup> However, whether other *Tenebrio* species have the same or even greater biodegradability and

plastic-degrading behavior, and if this ability is ubiquitous, remains unknown.

Currently, there are three extant *Tenebrio* species; two of them, *Tenebrio molitor* and *Tenebrio obscurus*, have been reported worldwide (Figure 1), while *T. opacus* Duftschmid, 1812 is only found in France.<sup>39–42</sup> Different from the *T. molitor*, the larvae of *T. obscurus* darken as they mature.<sup>43</sup> They are sold as a food source for pets in China and the U.S.A. Both species have been reported in North America, Asia, Europe, and Australia (Figure 1b). The adult beetle of *T. obscurus* appears similar to *T. molitor*, but the larvae of *T. obscurus* have dark black rings on the abdomen (Figure 1d). Mature *T. obscurus* larvae are similar in size (1.5–2.5 cm) to *T. molitor* larvae (Figure 1d, Figure S1c–f) and much smaller than *Z. morio* (up to 5.0–6.0 cm) (Figure S1a).<sup>44</sup> *T. obscurus* larvae are more sensitive to light and have higher cysteine in their proteins (by 15.6 times) than *T. molitor*.<sup>45</sup> The normal diet for commercial rearing of *T. obscurus* larvae is corn flour and oat, while *T. molitor* are fed with wheat bran.<sup>34</sup> We find that *T. obscurus* larvae obtained from China and the U.S.A. also chew and eat PS foam (Figure S1c–f).

In this study, we tested the hypothesis that PS degradation in members of the *Tenebrio* genus is ubiquitous by investigating the biodegradation by *T. obscurus* larvae obtained from an insect farm in Shandong Province, China, compared to that by *T. molitor*. We tested PS degradation using previously established protocols including (1) PS mass balance to determine the specific rates of PS degradation; (2) gel permeation chromatography (GPC) to assess changes in molecular weight; and (3) Fourier transform infrared (FTIR) spectroscopy of frass residues (excrement of larvae) to identify

chemical modifications resulting from PS digestion.<sup>1,34</sup> We also examined larval microbial communities using high-throughput sequencing before and after PS feeding. We found that *T. obscurus* larvae had the capacity to biodegrade PS at higher levels of depolymerization than the equally sized *T. molitor* larvae. PS degradation was also dependent on gut microbial community composition for both species, and Enterobacteriaceae showed a faster shift in relative abundance for *T. obscurus* than *T. molitor* larvae.

## MATERIALS AND METHODS

**Mealworm Sources and Feedstock.** In this study, *Tenebrio obscurus* larvae (approximately 2 cm in length) purchased from Zaozhuang Insects Breeding Plant, Shandong, and *Tenebrio molitor* larvae (approximately 2 cm in length) purchased from Binzhou Insects Breeding Plant, Shandong, China were utilized for PS biodegradation tests. Additional *T. obscurus* larvae were purchased to evaluate their ability to eat and chew PS foam from one U.S.A. source, Rocky Mountain Mealworms, Colorado, as well as from two sources in China: Guangyuan Insects Breeding Plant, Sichuan, and Luoyang Insects Breeding Plant, Henan (Figure S1).

The larvae for both species were identified based on morphology and coloration (Figure 1) as described by Robinson (2005) and Calmont and Soldati (2008).<sup>41,42</sup> Larvae of both species were not fed with any antibiotics according to suppliers. Prior to the tests, *T. molitor* larvae were fed with a normal diet of wheat bran while *T. obscurus* was fed with a normal diet of corn flour. Elementary ratios of C:H:O:N:S (w/w) were 35.7:6.5:41.5:2.7:0.5 for bran and 38.5:7.4:48.0:1.2:0.3 for corn flour. Both diets provide sufficient nutrients as well as a source of carbon for mealworm growth.

Expanded PS foam (or Styrofoam as commercial name) was used as feedstock for the larvae of both species and was purchased from Lan Tian Plastic Company, Guangdong, China. The PS feedstock contained polystyrene purity over 96.3%. Number-average molecular weight ( $M_n$ ) of the PS was 107000 and weight-average molecular weight ( $M_w$ ) was 345000 with a density of  $0.0197 \pm 0.0009 \text{ g/cm}^3$  and a water angle of  $104.2 \pm 2.1^\circ$ . No extra additives or catalysts were added, according to the manufacturer. According to our analysis, the bromide content in the PS foam was under the detection limit ( $<1 \text{ mg g}^{-1}$ ), indicating that there are no brominated flame retardants in it. The tetrahydrofuran (THF, GC grade purity  $\geq 99.9\%$ ), gentamicin sulfate, and trypticase soy agar (TSA) were purchased from Aladdin, Shanghai, China.

### Mealworm Survival Rates and Biodegradation of PS.

To compare the PS consumption and biodegradation between the larvae of the two species, six treatments were prepared based on feeding conditions: starvation of *T. molitor*, PS-fed *T. molitor*, PS plus bran-fed *T. molitor*, starvation of *T. obscurus*, PS-fed *T. obscurus*, and PS plus corn flour-fed *T. obscurus*. Wheat bran or corn flour can provide sufficient nutrients for their growth. For each treatment, initial larvae (410) were randomly selected and placed in a food grade polypropylene container (volume of 3300 mL) under controlled conditions ( $25 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  humidity, and dark environment). To assess PS consuming capacity initially, PS blocks (7.2 g) were added. Co-diet treatments were PS plus bran (1.2 g) for *T. molitor* larvae and PS plus corn flour (1.2 g) for *T. obscurus* larvae. An additional 1.2 g of the codiet was supplemented

every 5 days to reach a final ratio of PS to codiet of 1.0:1.0 at the end of test. This ratio was the same to that previously used to test the effect of the codiet on PE and PS degradation in *T. molitor* larvae.<sup>35</sup> Prior to the test, larvae were fed their regular food for at least 3 days, then they were fasted for 1 day, and finally were fed with respective feedstocks.

The measurement of survival rates and plastic mass loss was carried out every 5 days and ended on day 31. We selected a 31 day period to avoid pupation, and a high survival rate of *T. molitor* larvae could be maintained within 4–5 weeks with PS as the only diet.<sup>34</sup> During the measurement time, dead larvae and molted exoskeletons were removed from containers to prevent their being eaten by the remaining larvae since cannibalism existed in the later period.<sup>33</sup> All treatments were conducted in duplicate.

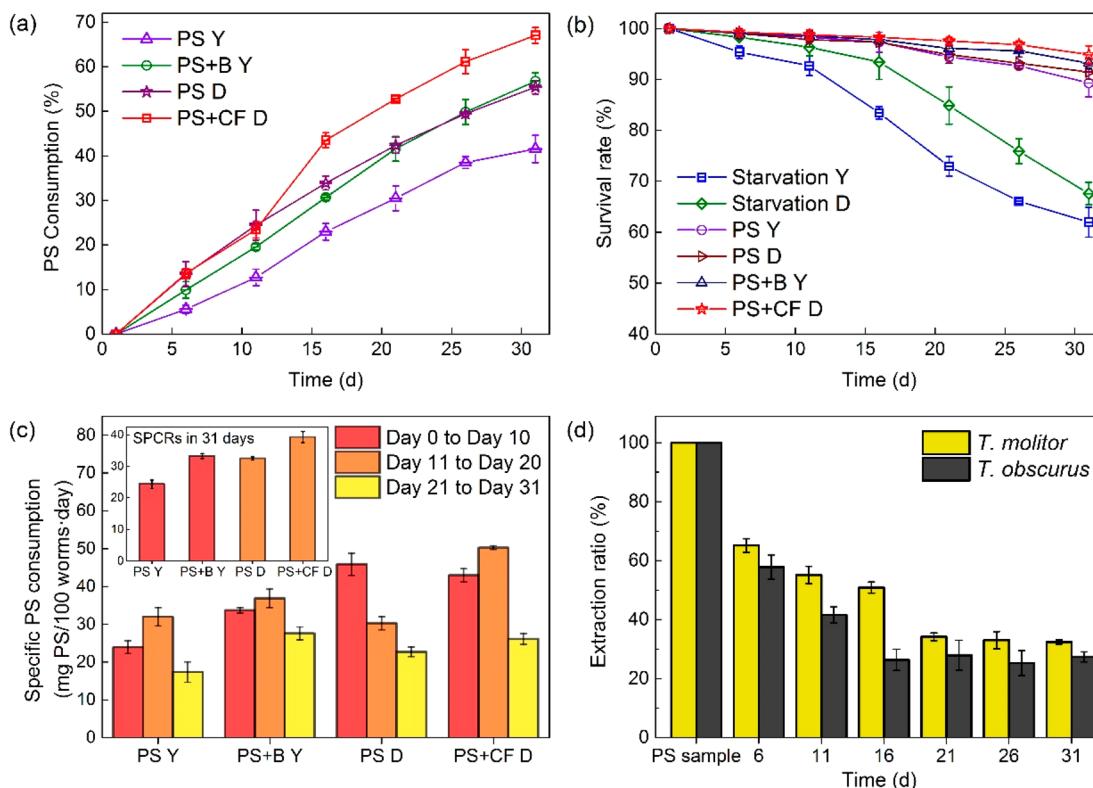
**THF Extraction.** THF extraction was used to determine the mass of PS residue in frass during the test period as described previously.<sup>34,35</sup> The egested frass contained an extractable part and a nonextractable part; the extractable part consisted of the undigested PS polymer and modified PS polymers, while the nonextractable part consisted of other residues, such as undigested exoskeletons.<sup>34,35</sup> After being dried at ambient temperature, frass samples (0.5 g) were extracted with 10 mL of THF for 3 h with a magnetic stirrer. Then, the extracted THF solution was filtered with a 0.22  $\mu\text{m}$  poly(ether sulfone) (PES) membrane filter and collected in a preweighed glass vial. After evaporation, polymer residue in the vial was weighed to calculate the mass of THF-extracted polymer.<sup>34,35</sup> All THF extraction tests were conducted in triplicate.

The THF extractable ratio (TER) was used as an indicator of PS biodegradation or digestion in larval guts and was calculated on the basis of the weight of extracted polymer divided by the weight of the sample for THF extraction.<sup>34</sup> PS feedstock showed 100% while TER of frass depended on the content of residual or not-degraded PS.

**Frass Collection and Analytical Methods.** To obtain enough frass for characterization, additional larvae (approximately 2000 for each group) were raised as described above. Likewise, mass of PS and codiets were increased proportionately. At the end of the 31 day test, larvae were cleansed by streambed air and transferred to a clean iron container to collect frass within 24 h. The collected frass was immediately stored at  $-20^\circ\text{C}$  for final characterization. The morphology of frass of PS-fed *T. molitor* and *T. obscurus* larvae was observed by SEM-EDX (SI M2, Figures S3 and S4).

Gel permeation chromatography (GPC) (Agilent 1260, Agilent Technologies Inc., U.S.A.) was applied to analyze changes of molecular weight of the polymer. Preparation of samples was similar to THF extraction test described previously. PS was extracted from PS feedstock (1.0 g) and frass samples (1.0 g) from PS-fed larvae by THF. After filtration, the THF solution was mixed on a magnetic stirrer with gentle heating ( $60^\circ\text{C}$ ). After the extracted solution was concentrated to 5 mL in volume, the extract (20  $\mu\text{L}$  in volume) was injected into the GPC analyzer, with a flow rate of 0.8 mL/min.<sup>1,34,35</sup>

Fourier transform infrared spectroscopy (FTIR) (Nicolet iS05 FTIR Spectrometer, Thermo Fisher Scientific, U.S.A.) was applied to characterize major functional groups of PS feedstock sample and frass in the range of 4000–500  $\text{cm}^{-1}$ . Prior to the analyses, samples were dried in a freeze drier for at least 36 h and then ground with KBr to prepare a homogeneous KBr pellet for scanning.<sup>1,32–34</sup>



**Figure 2.** Comparison of PS digestion by *Tenebrio obscurus* and *Tenebrio molitor* larvae. (a) PS foam consumption with PS as the sole diet and PS plus the codiet corn flour for *T. obscurus*, and PS as the sole diet and PS plus bran for *T. molitor*. (b) Survival rates for both species fed with PS only and PS plus the codiet versus starvation control. (c) Specific PS consumption rates of *T. obscurus* fed with PS only and PS plus corn flour versus *T. molitor* fed with PS only and PS plus bran during days 0 to 10, days 11 to 20, and days 21 to 31. The average specific rates are illustrated in the inset figure. (d) Progressive decrease in the THF-extractable fraction in the frass of *T. obscurus* and *T. molitor* fed with PS foam only. The test lasted 31 days with 410 mealworms in each incubator and 7.2 g PS foam. Y = *T. molitor*; D = *T. obscurus*; B = bran; CF = corn flour; PS = polystyrene.

Thermal gravimetric analysis (TGA) (TA-Q500, TA Instruments, U.S.A.) was applied to characterize thermal changes from PS to frass. The heating program included two different atmospheres so as to study pyrolysis of the sample under a nitrogen and air ambience, respectively. Samples (5 mg) were heated from 40 to 800 °C at a rate of 20 °C/min under a high-purity nitrogen ambience (99.999%), then cooled to 500 °C, and finally heated again to 800 °C under an air ambience.

**Antibiotic Suppression Test.** The effect of antibiotic suppression on PS depolymerization and degradation was tested using *T. obscurus* larvae with gentamicin as an inhibitor. Gentamicin was selected based on previous results.<sup>32,33</sup> The gentamicin suppressive group (410 larvae) was fed antibiotic feedstock (AF) containing corn flour versus gentamicin sulfate at 100:3 (w/w) for 3 days and then fed with PS feedstock. AF was continuously fed with PS feedstock and resupplied every 3 days. On day 15, frass samples from the antibiotics treatment were collected to analyze for the molecular weights using GPC.

On day 0 (prior to feeding PS), 7, and 15, 15 *T. obscurus* larvae were randomly chosen from each source of larval populations to prepare a gut suspension for counting of gut microorganisms. The larvae were decontaminated by 80% ethanol, rinsed by Milli-Q water, and anatomized to obtain the guts. Subsequently, gut contents were extracted and suspended in 5 mL sterile saline water, serial diluted (10<sup>-1</sup> to 10<sup>-7</sup>), and cultivated on nonselective TSA plates at 37 °C for 24 h. Finally, the number of colonies were counted as described elsewhere.<sup>33</sup>

**Microbial Community Analysis.** Larvae used for microbial community analysis were collected from PS-fed *T. obscurus* and PS-fed *T. molitor* treatments at day 0, day 6, and day 11 for microbial community analysis. The DNA extraction and PCR amplification methods were similar to that reported previously and are detailed in Supporting Information (SI M3).

Phasing amplicon sequencing was applied to sequence the V3–V4 region of the 16S rRNA gene. Purified amplicons were paired-end sequenced on an Illumina MiSeq platform. Sequencing data was demultiplexed, quality-filtered on Trimmomatic, and merged according to criteria.<sup>46</sup> Operational Taxonomic Units (OTUs) were clustered with 0.97 identity threshold using UPARSE.<sup>47</sup> Chimeric sequences were identified and removed using UCHIME. Taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier against the Silva 16S rRNA database with a confidence threshold of 70%. Finally, microbial community composition, hierarchical cluster analysis, principal coordinate analysis (PCoA), and ternary analysis were run on the free online platform of the Majorbio I-Sanger Cloud Platform (Shanghai, China).

## RESULTS AND DISCUSSION

### PS Consumption of *T. obscurus* vs *T. molitor* larvae.

All *T. obscurus* larvae purchased from Shandong, Sichuan and Henan Provinces, China and Colorado, U.S.A. chewed and ate PS foam (Figures 1 and S1). We surmise that the chewing and ingestion of PS foam is likely an adaptive behavior intrinsic to *T. obscurus*. They behaved similarly to each other but

**Table 1.** Summary of PS Biodegradation by *T. obscurus* and *T. molitor* Larvae

Mealworm source	Initial weight, mg larva <sup>-1</sup>	Feed	Weight change (%) at the end of test	Survival rate %	Specific PS consumption rate <sup>a</sup>	
					mg PS 100 larvae <sup>-1</sup> d <sup>-1</sup>	mg PS g larvae <sup>-1</sup> d <sup>-1</sup>
<i>T. obscurus</i>	66.15 ± 2.13	PS+C	+15.9 ± 4.1	94.9 ± 1.7	39.24 ± 1.73	5.94 ± 0.26
		PS	-8.1 ± 3.7	91.5 ± 1.5	32.44 ± 0.51	5.06 ± 0.08
		Unfed	-13.2 ± 2.7	67.6 ± 2.2	nd	nd
<i>T. molitor</i>	62.41 ± 1.72	PS+B	+14.6 ± 1.8	93.2 ± 1.0	33.23 ± 0.80	5.33 ± 0.13
		PS	-8.6 ± 1.2	89.3 ± 2.7	24.30 ± 1.34	3.89 ± 0.47
		Unfed	-18.2 ± 6.0	62.0 ± 2.9	nd	nd

<sup>a</sup>Specific PS consumption rates were calculated on the basis of the mass of PS consumed over the test period (31 days). PS = polystyrene; C = corn flour; B = bran; nd = not determined. Test temperature was at 25 °C.

differently from *T. molitor* larvae. They were all sensitive to light and mostly hid below PS foam in clusters (Figure 1d, Figure S1 c–f). The larvae of *T. molitor* were less sensitive to light and spread themselves on the foam surface or penetrated the inside matrix (Figure 1a,c). The *T. obscurus* larvae purchased from Shandong, China were selected for further study because of stable supply. The PS consumption by both species increased progressively (Figure 2a). The *T. obscurus* were capable of rapid PS consumption at rates which were even greater than those of *T. molitor*. During the 31 day test with PS as the only diet, the PS mass consumption by the *T. obscurus* larvae was 55.4% ± 1.5% while that by *T. molitor* was 41.5% ± 3.0% (Figure 2a). The PS consumption increased when codiets were added, i.e., the *T. obscurus* consumed 67.1% ± 1.8% of PS and *T. molitor* consumed 56.8% ± 1.9%. The specific rate of PS consumption was calculated as the amount of ingested PS per day for 100 mealworms based on the mass loss of PS foam, and as average numbers of survived mealworms.

At the end of the 31 day test at 25 °C, the survival rates (SRs) of both species fed EPS alone were 91.5 ± 1.5% and 89.3 ± 2.7%, respectively, significantly greater than those of unfed controls (67.6 ± 2.2% and 62.0 ± 2.9%) (Table 1) and not significantly less than corn flour-fed and bran-fed larvae (95.0 ± 1.7% and 93.2 ± 1.0%) (Table 1 and Figure 2b). Over the 31 day test period, starved larvae of both species lost 13.2 ± 2.7% and 18.2 ± 6.0% of their average weights, respectively. As expected, the larvae of *T. obscurus* and *T. molitor* fed with PS alone lost their average weight slightly by 8.1 ± 3.7% and 8.6 ± 1.2%, respectively, while those fed with corn flour and bran experienced a 15.9 ± 4.1% and 14.6 ± 1.8% weight gain, respectively (Table 1). As expected, the survival rates of both starvation species were much lower than those fed with PS only and PS plus codiet (corn flour or bran) at the end of the 31 day period, as observed previously with *T. molitor*.<sup>33</sup> The *T. obscurus* larvae showed slightly better survival than *T. molitor* (Figure 2b). The observation supported the fact that the addition of the nutrition-rich codiet enhances PS consumption by both species.<sup>34,35</sup> When PS was applied as the sole diet, the higher SRs and slight decrease in average weights of both species indicated that larvae received their energy source from digestion of PS but lacked a nutrition source for their growth, as observed previously.<sup>33</sup>

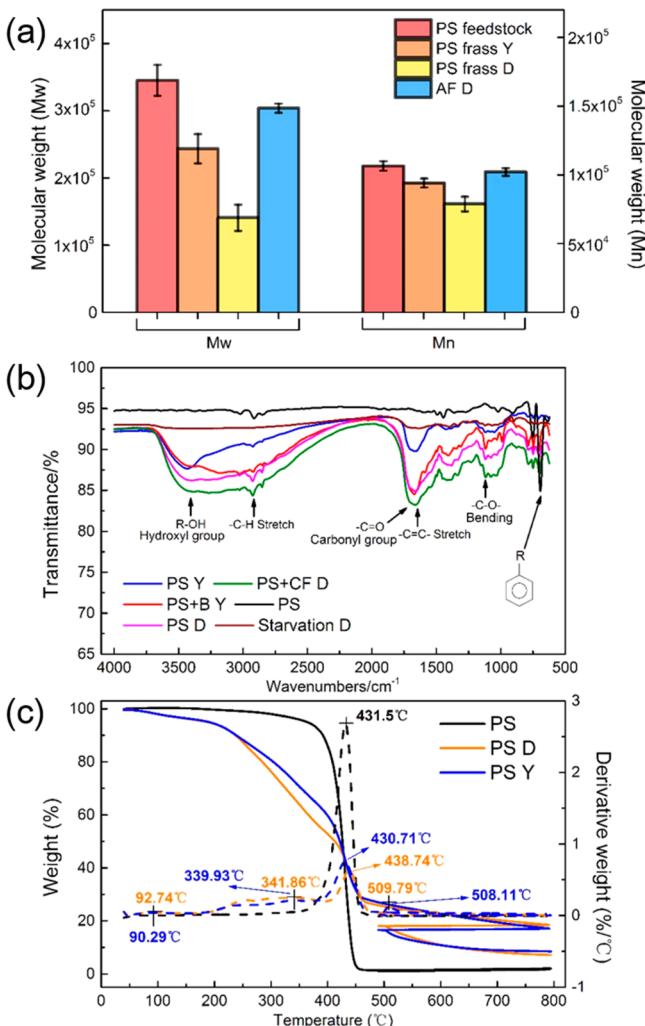
The specific PS consumption rates (SPCRs) were calculated for days 0 to 10, 11 to 20, and 21 to 31 (Figure 2c) and for the overall 31 day period (Figure 2c inset). Overall, *T. obscurus* larvae exhibited comparatively greater PS consumption ability than *T. molitor*, i.e., 32.44 ± 0.51 versus 24.30 ± 1.34 mg 100 worms<sup>-1</sup> d<sup>-1</sup> fed with PS only and 39.24 ± 1.73 versus 33.23 ±

0.80 mg 100 worms<sup>-1</sup> d<sup>-1</sup> when codiet corn flour and bran were fed, respectively (Table 1). The rates are within the rate ranges of 12 *T. molitor* larvae collected from China, U.S.A., and UK, i.e., 8.5–21 mg 100 worms<sup>-1</sup> d<sup>-1</sup> for *T. molitor* fed with PS only and 24–46 mg 100 worms<sup>-1</sup> d<sup>-1</sup> for the worms fed with PS plus codiet.<sup>33</sup> The results also confirmed that higher SPCR can be achieved in *T. obscurus* when the nutrition-rich codiet is supplied, as reported with the tests with *T. molitor*.<sup>34,35</sup> The results of this study also showed that SPCRs were increased during the initial 21 days and then declined (Figure 2c). Further research is needed to understand whether this was due to maturation of the larvae or other unknown reasons.

Change of TER, i.e., THF extractable ratio (which represents the fraction of residual PS polymer in egested frass), can indirectly indicate the change of PS degradability in mealworm gut.<sup>34</sup> The TER of frass from both species (Figure 2d) fed with PS only decreased during the 31 day test from 57.8% ± 4.1% and 65.2% ± 2.3% on day 6 progressively and finally stabilized at 27.3% ± 1.7% and 32.4% ± 0.7%, respectively, a value that was significantly different ( $p < 0.01$ ). The decrease of TERs during the 31 day period indicated that PS degradation and then mineralization in the gut of larvae increased gradually, likely due to an increase in PS-degrading microbial activities. In addition, the final TER of the frass of *T. obscurus* was significantly lower than that of *T. molitor* ( $p < 0.01$ ). Therefore, we concluded that *T. obscurus* was superior to *T. molitor* for PS biodegradability.

#### Biodegradability and Evidence of Depolymerization.

GPC analysis was conducted at the end of the 31 day test to characterize the depolymerization and biodegradation of ingested PS using the established methods.<sup>1,34</sup> Frass samples from the *T. obscurus* fed with PS only contained polymer extracts with  $M_n$  values that were 26.0% lower than the feedstock and  $M_w$  values that were 59.2% lower than the feedstock (PS feedstock with  $M_n$  of 107000;  $M_w$  of 345000). Frass samples from *T. molitor* had  $M_n$  values that were 11.7% lower and  $M_w$  values that were 29.8% lower than the feedstock (Figure 3a). These decreases in  $M_n$  and  $M_w$  were significant for all sources (*t* test,  $p < 0.05$ ), indicating depolymerization and degradation of PS feedstock was ubiquitous across both species. The result indicated that *T. obscurus* larvae had superior PS depolymerization and biodegradation than *T. molitor*. In addition, except for the macromolecular peak, some low-molecular-weight peaks (molecular weights between 200 and 1400) were also detected in the frass samples from *T. obscurus* and *T. molitor* fed with PS only, suggesting that some oligomer products might be generated (Table S3). Further



**Figure 3.** Depolymerization of PS by *Tenebrio obscurus* and *Tenebrio molitor* larvae. (a) Comparison of  $M_w$  and  $M_n$  of PS feedstock, PS polymers extracted from frass of *T. molitor* fed PS only (PS frass Y), *T. obscurus* fed with PS only (PS frass D), and *T. obscurus* fed with PS only in the presence of gentamicin (AF D). (b) FTIR spectra of PS feedstock and the frass of *T. molitor* and *T. obscurus* fed PS only or PS plus the codiet in comparison with frass of *T. obscurus* under starvation. (c) TGA spectra of PS feedstock and frass of *T. molitor* and *T. obscurus* larvae fed with PS only. Weight curve in solid line (left axis). Derivative weight curve in dash line (right axis). Y = *T. molitor*; D = *T. obscurus*; AF = antibiotics; B = bran; CF = corn flour; PS = polystyrene.

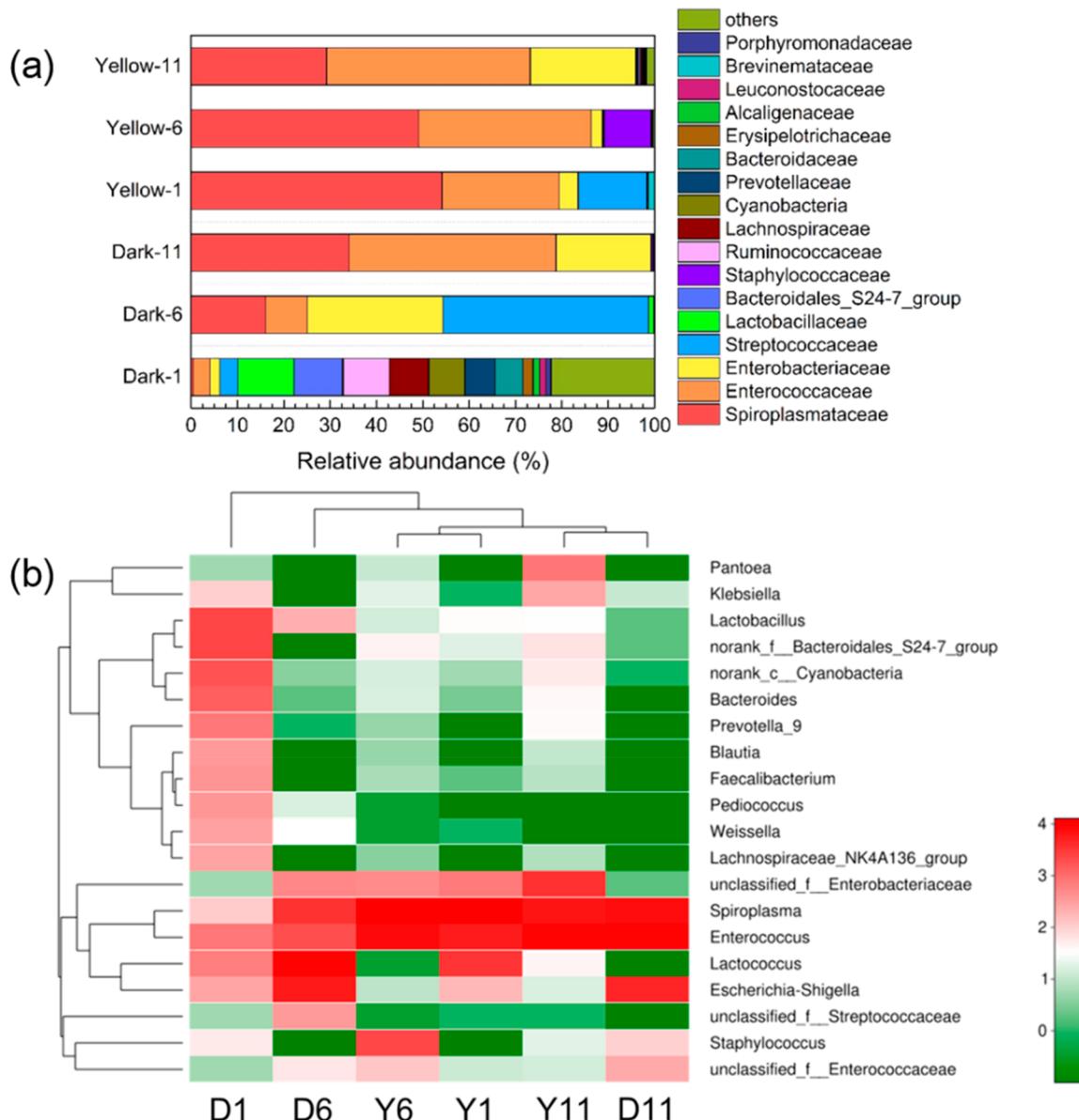
study is needed to identify these compounds and PS degraded intermediates.

Evidence of oxidation and depolymerization of the PS polymer was also obtained using FTIR analysis at the end of the 31 day test. A comparison of FTIR spectra for the PS feed stock sample and egested frass of both species showed chemical changes and incorporation of oxygen previously linked to plastic degradation (Figure 3b). The FTIR data with appearance of carbonyl groups are similar to the data showing PS biodegradation in *T. molitor* larvae previously reported.<sup>1,32–35</sup> The spectra of frass from *T. obscurus* fed with PS and PS plus corn flour were similar to those from *T. molitor* fed with PS and PS plus bran and were significantly different from the frass of unfed *T. obscurus* and PS feedstock (Figure 3b). Peaks at 625–970 cm<sup>-1</sup> (ring-bending vibration) had high

intensities in the PS feedstock but were much weaker in frass samples of both species, indicating a decrease of PS polymer in frass samples. Peaks characteristic of the PS benzene ring (C=C stretch, 1550–1610 and 1800–2000 cm<sup>-1</sup>) were damped in frass of larvae fed PS or PS plus corn flour or bran, suggesting ring cleavage. In the frass from *T. obscurus* fed PS, new functional groups were observed, i.e., peaks at 1075–1150 cm<sup>-1</sup> (–C–O–), 1650 cm<sup>-1</sup> (–C=C– stretch), and 1700 cm<sup>-1</sup> (–C=O stretch), suggesting that the oxidation and depolymerization processes of PS which occurred in *T. obscurus* were the same as those occurring in *T. molitor*. The addition of the oxygen functional groups into the polymer chain was considered as the preliminary and key step in plastic degradation in previous studies.<sup>25,48</sup> Moreover, the broad peaks at about 3440 cm<sup>-1</sup> were assigned to hydroxyl or carboxylic acid groups. This suggested that the surface had a change from a hydrophobic to a more hydrophilic property. FTIR analysis confirmed that all frass samples showed strong signs of PS biodegradation, as observed in previous tests of *T. molitor* from Beijing,<sup>1</sup> a California PetCo store,<sup>34</sup> plus 12 other larval populations around the world,<sup>33</sup> and demonstrated the depolymerization and biodegradation capabilities of *T. obscurus* tested in this study.

Thermal modifications of ingested PS were detected using TGA at the end of the 31 day test. As shown in Figure 3c, only one maximum decomposition rate (about 435 °C) was detected in the PS sample. In contrast, for frass from *T. obscurus* fed PS only (PS D) and from *T. molitor* (PS Y), four maximum decomposition rates (three under a N<sub>2</sub> ambience and one under an air ambience) appeared at about 92.74, 341.86, 438.74, and 509.79 °C, respectively (Figure 3c). The decomposed part under 100 °C was possibly classified as volatile organics (gut secretion, carboxylic acid compounds from PS biodegradation, etc.) while decomposed parts from 100 to 360 °C might be attributed to other biological wastes and biodegradation residue. The frass from both species decomposed in same way, suggesting the production of new organic intermediates with different thermal properties in the guts of the larvae.<sup>1</sup> The mass loss ratio of the frass of *T. obscurus* larvae in the stage of 360 to 480 °C was 35.15% while that of *T. molitor* larvae was 41.03%, in comparison with the PS feedstock of 96.32%. This result implied that the PS polymer structure deteriorated as it passed through the guts and that more PS was depleted or biodegraded in *T. obscurus*, suggesting that larvae of *T. obscurus* were more efficient in PS biodegradation than the larvae of *T. molitor*.

**Antibiotics Suppression Test.** A gentamicin suppression test was performed to identify the role of gut bacteria of *T. obscurus* in PS biodegradation. The number of gut bacteria in *T. obscurus* larvae receiving gentamicin sharply decreased from  $0.86 \times 10^6$  to  $5.7 \times 10^4$  and  $4.4 \times 10^4$  CFU per gut by almost two to three magnitudes after receiving gentamycin for 7 or 15 days. Meanwhile, the controls without antibiotics remained unchanged at the level near  $10^7$  CFU per gut (Figure S5 and Table S4), implying that the gut bacteria were effectively suppressed by gentamicin sulfate. On day 15, the frass was collected for GPC analysis. The  $M_w$  and  $M_n$  values were basically unchanged (Figure 3a), demonstrating that PS depolymerization was almost completely inhibited in the presence of gentamicin. When gut bacteria were inhibited, the *T. obscurus* completely lost the capacity of PS depolymerization and thus, biodegradation. This was similar to observations where PS depolymerization and then biodegradation by *T.*



**Figure 4.** Gut microbial community analysis of *T. obscurus* versus *T. molitor* larvae prior to test (D1 and Y1) and fed with PS only on day 6 and day 11 (D6, D11 versus Y6 and Y11). (a) Relative bacterial abundances of *T. obscurus* and *T. molitor* larvae fed with PS only at family level. (b) Hierarchical cluster analysis of gut bacterial communities of *T. obscurus* and *T. molitor* larvae prior to test and fed with PS only. The analysis was on the basis of top 20 abundant genera in the six samples. The color intensity of the scale indicates the relative abundance. Y = Yellow = *T. molitor*; D = Dark = *T. obscurus*.

*molitor* were inhibited by supplementation with the antibiotic gentamicin.<sup>32,33</sup> Therefore, we can conclude that PS biodegradation by *Tenebrio* larvae is gut microbe dependent. Herein, we further hypothesize that the depolymerization of PS may be attributed to the direct biochemical effect of gut bacteria or a combined effect with the larval digestive system (e.g., enzymatic, physical and chemical reactions). *T. obscurus* chews plastic foam into small fragments and increases the surface area of the polymers, which benefits depolymerization by extracellular enzyme(s).<sup>48,49</sup> The efficient biodegradation of PS is achieved via a synergistic effect of the activities of larvae of *Tenebrio* and their gut microbes.

**Gut Microbial Analysis.** Microbial community analysis using Illumina MiSeq indicated that the gut microbiomes of the larvae of both species shifted significantly after PS was fed as the only diet. In this study, the mealworms prior to PS feeding

were named as D1 and Y1 (D = *T. obscurus*; Y = *T. molitor*), while D6, Y6, D11, Y11 represented the PS-fed mealworms on day 6 and day 11, respectively. A total of 233596 sequences were obtained, with an average length of 448.193 bps, and sampling coverage was above 0.99, suggesting that Illumina MiSeq sequencing was capable of detecting most of the reads.<sup>50</sup>

Rarefaction curves with a Shannon Index at the operational taxonomic unit (OTU) level were generated for all samples at 0.97 identity and reflected that D1 had highest taxonomic richness, while other samples showed similar richness (Figure S6a). The rarefaction curves of all samples achieved a plateau at about 4000 reads, implying that the amplicons were enough for sequencing. The results indicated that the gut microbiome had higher diversity in *T. obscurus* prior to feeding PS in comparison with *T. molitor*. The difference in initial diversity could be related to their normal diets and feed history, as well

as gut eco-physiology. After ingesting PS, there was a decrease in microbial community diversity in *T. obscurus* but an increase for *T. molitor* (Figure S6a). This change of microbial structure was investigated using Principal coordinate analysis (PCoA) at the OTU level (Figure S6b). The microbial community structure for both species approached similarity on day 11.

On the basis of relative abundance, the gut microbiome of these two *Tenebrio* species mainly consisted of 17 families (Figure 4a). Spiroplasmataceae, Enterococcaceae, Enterobacteriaceae, and Streptococcaceae are the dominant families. After PS feeding, community shift was mainly associated with the distribution of Spiroplasmataceae, Enterococcaceae, and Enterobacteriaceae. For instance, the ingestion of PS resulted in the relative abundance increase of Enterobacteriaceae in *T. obscurus* from 2.09% to 29.21% and 20.52% on day 6 and day 11, respectively. Meanwhile, the Enterobacteriaceae reached relatively higher abundance more quickly in *T. obscurus* than in *T. molitor*. Previous work by Brandon et al. (2018) found that *Citrobacter* sp. and *Kosakonia* sp., which were demonstrated strongly, associated with PE and PS degradation by *T. molitor* and belonged to the family Enterobacteriaceae.<sup>35</sup> Furthermore, PE degrading *Enterobacter absuriae* YT1 isolated from the gut of the Indian meal moth (larvae of *Plodia interpunctella*) also belonged to the family Enterobacteriaceae.<sup>51</sup> The increase in the Enterobacteriaceae likely related to the development of PS-degrading microbes. The results suggest that the gut microbial community of *T. obscurus* shifted to that of high PS degradation capacity compared to *T. molitor*. The relative abundances of the families of Spiroplasmataceae and Enterococcaceae also changed noticeably in both species, which are known gut-associated bacteria in the *Tenebrio* gut microbiome.<sup>52,53</sup> A ternary analysis suggested that the families Enterococcaceae, Spiroplasmataceae, and Enterobacteriaceae were strongly associated with the PS diet in *T. obscurus* (Figure S7), which was consistent with the result in relative abundance distributions.

To further compare differences in gut microbial communities of both species, hierarchical clustered heatmap analysis was conducted at the genus level (Figure 4b).<sup>54,55</sup> As Figure 4b shows, four clusters were generated from six samples: Cluster I (Dark-11 and Yellow-11), Cluster II (Yellow-1 and Yellow-6), Cluster III (Dark 6), and Cluster IV (Dark 1). Among the four clusters, microbial communities in Cluster I exhibited high similarities, implying a homology of microbial community structure in both species that were fed PS after 11 days. However, Cluster III and Cluster I were separated from Cluster IV, which further verified that the ingestion of PS could shift the gut microbial structure, and *T. obscurus* were more adapted to the PS diet. Further study will address the key functional microorganisms which play an essential role in PS depolymerization and their synergistic relation with *Tenebrio* larvae if it is present.

**Implications.** This work is the first report to demonstrate that PS, one of the most highly persistent plastics, can be depolymerized and undergoes chemical modifications within the gut of larvae of *T. obscurus*. We demonstrated for the first time that not only *T. molitor* but also *T. obscurus* may have ubiquitous capabilities to degrade PS, which supports our hypothesis that the ability of insects to depolymerize PS and other plastics is not restricted to *T. molitor*.<sup>32,33</sup> In this study, *T. obscurus* larvae from sources in China and the U.S.A. exhibited an ability to chew and ingest PS; the selected *T. obscurus* larvae for PS degradation tests demonstrated higher

consumption rates and depolymerization extents than *T. molitor* larvae. Although further investigation of other sources of *T. obscurus* is needed to provide supplementary convincing evidence and mechanisms of the ubiquity of PS biodegradation in the family members belonging to darkling beetles (Coleoptera: Tenebrionidae), the larvae of *T. obscurus* and *T. molitor* are likely candidates of plastics-degrading organisms. Our work revealed that the PS biodegradability of the *Tenebrio* genus members is gut microbe dependent, suggesting that a plastic-degrading ecosystem exists and/or has been under development via bioactivities on multiple levels. Future work is also needed to confirm whether *T. obscurus* has the capacity to degrade other common plastics such as PE, PP, PVC, and PET as well as to confirm the mechanisms and pathways of such biodegradation. At present, it is still difficult to imagine that mealworms could be utilized directly for a solution toward the global crisis of plastic pollution. Since the gut microbiome of the larvae is the key in biodegradation, more research should be done on replicating the gut process and conditions through bacterial cultures. More efforts in scientific research based on our studies and our findings on the degradation abilities of the *Tenebrio* genus could inspire a technological approach to solve the problems of plastic waste and microplastic pollution.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.est.8b06963](https://doi.org/10.1021/acs.est.8b06963).

Morphology of larvae of *Z. morio*, *T. molitor*, and *T. obscurus* from Shandong, Sichuan, and Henan, China, and Colorado, U.S.A.; bran-feeding pre-experiment for *T. obscurus*; SEM image and EDX mapping of frass from PS-fed *T. molitor*; SEM image and EDX mapping of frass from PS-fed *T. obscurus*; gentamicin suppression test on gut bacteria; rarefaction curves of the Shannon index and PCoA analysis; ternary analysis of gut bacteria in *T. obscurus* at day 1, day 6, and day 11; element composition of the codiets; partial elementary scanning of frass from PS-fed dark and *T. molitor* by EDX; molecular weight of all peaks detected from samples by the GPC analysis; CFUs of gut microbes of *T. obscurus* during antibiotics suppression test; overview of samples information; sequence data are available with the BioProject number SRP186213 on NCBI ([PDF](#))

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### Notes

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## ■ REFERENCES

- (1) Yang, Y.; Yang, J.; Wu, W. M.; Zhao, J.; Song, Y.; Gao, L.; Yang, R.; Jiang, L. Biodegradation and Mineralization of Polystyrene by Plastic-Eating Mealworms: Part 1. Chemical and Physical Characterization and Isotopic Tests. *Environ. Sci. Technol.* **2015**, *49* (20), 12080–6.
- (2) Jambeck, J. R.; Geyer, R.; Wilcox, C.; Siegler, T. R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K. L. Marine pollution. Plastic waste inputs from land into the ocean. *Science* **2015**, *347* (6223), 768–771.
- (3) Law, K. L.; Morét-Ferguson, S.; Maximenko, N. A.; Proskurowski, G.; Peacock, E. E.; Hafner, J.; Reddy, C. M. Plastic accumulation in the North Atlantic subtropical gyre. *Science* **2010**, *329* (5996), 1185.
- (4) Bergmann, M.; Sandhop, N.; Schewe, I.; D'Hert, D. Observations of floating anthropogenic litter in the Barents Sea and Fram Strait, Arctic. *Polar Biol.* **2016**, *39* (3), 553–560.
- (5) Engler, R. E. The Complex Interaction between Marine Debris and Toxic Chemicals in the Ocean. *Environ. Sci. Technol.* **2012**, *46* (22), 12302.
- (6) Li, L. I.; Tse, H. F.; Fok, L. Plastic waste in the marine environment: A review of sources, occurrence and effects. *Sci. Total Environ.* **2016**, *566–567*, 333–349.
- (7) Elsawy, M. A.; Kim, K. H.; Park, J. W.; Deep, A. Hydrolytic degradation of polylactic acid (PLA) and its composites. *Renewable Sustainable Energy Rev.* **2017**, *79*, 1346–1352.
- (8) Oberbeckmann, S.; Löder, M.; Labrenz, M. Marine microplastic-associated biofilms - A review. *Environmental Chemistry* **2015**, *12* (5), 551–562.
- (9) Vandermeersch, G.; Van Cauwenbergh, L.; Janssen, C. R.; Marques, A.; Granby, K.; Fait, G.; Kotterman, M. J.; Diogene, J.; Bekaert, K.; Robbins, J.; Devriese, L. A critical view on microplastic quantification in aquatic organisms. *Environ. Res.* **2015**, *143*, 46–55.
- (10) Mintenig, S. M.; Int-Veen, I.; Loder, M. G. J.; Primpke, S.; Gerdts, G. Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. *Water Res.* **2017**, *108*, 365–372.
- (11) Wang, W.; Ndungu, A. W.; Li, Z.; Wang, J. Microplastics pollution in inland freshwaters of China: A case study in urban surface waters of Wuhan, China. *Sci. Total Environ.* **2017**, *575*, 1369–1374.
- (12) Diepens, N. J.; Koelmans, A. A. Accumulation of plastic debris and associated contaminants in aquatic food webs. *Environ. Sci. Technol.* **2018**, *52*, 8510–8520.
- (13) Weber, A.; Scherer, C.; Brennholt, N.; Reifferscheid, G.; Wagner, M. PET microplastics do not negatively affect the survival, development, metabolism and feeding activity of the freshwater invertebrate *Gammarus pulex*. *Environ. Pollut.* **2018**, *234*, 181.
- (14) PlasticsEurope, 2016/10/20. Plastics—the Facts 2016; p. 534. <http://www.plasticseurope.org/Document/plastics—the-facts-2016-15787.aspx?Fol=535> ID.
- (15) Zhou, P.; Huang, C.; Fang, H.; Cai, W.; Li, D.; Li, X.; Yu, H. The abundance, composition and sources of marine debris in coastal seawaters or beaches around the northern South China Sea (China). *Mar. Pollut. Bull.* **2011**, *62* (9), 1998–2007.
- (16) Hidalgo-Ruz, H.-R.; L, G.; RC, T.; M, T. Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environ. Sci. Technol.* **2012**, *46* (6), 3060–3075.
- (17) Wu, W. M.; Yang, J.; Criddle, C. S. Microplastics pollution and reduction strategies. *Front. Environ. Sci. Eng.* **2017**, *11* (1), 6.
- (18) Lee, B.; Pometto, A. L.; Fratzke, A.; Bailey, T. B. Biodegradation of degradable plastic polyethylene by *phanerochaete* and *streptomyces* species. *Appl. Environ. Microbiol.* **1991**, *57* (3), 678–685.
- (19) Jones, P. H.; Prasad, D.; Heskins, M.; Morgan, M. H.; Guillet, J. E. Biodegradability of photodegraded polymers. I. Development of experimental procedures. *Environ. Sci. Technol.* **1974**, *8* (10), 919–923.
- (20) Guillet, J. E.; Regulski, T. W.; Mcaneney, T. B. Biodegradability of photodegraded polymers. II. Tracer studies of biooxidation of Ecotype PS polystyrene. *Environ. Sci. Technol.* **1974**, *8* (10), 923–925.
- (21) Amass, W.; Amass, A.; Tighe, B. A review of biodegradable polymers: uses, current developments in the synthesis and characterization of biodegradable polyesters, blends of biodegradable polymers and recent advances in biodegradation studies. *Polym. Int.* **1988**, *47* (2), 89–144.
- (22) Kyrikou, I.; Briassoulis, D. Biodegradation of Agricultural Plastic Films: A Critical Review. *J. Polym. Environ.* **2007**, *15* (2), 125–150.
- (23) Austin, H. P.; Allen, M. D.; Donohoe, B. S.; Rorrer, N. A.; Kearns, F. L.; Silveira, R. L.; Pollard, B. C.; Dominick, G.; Duman, R.; El, K. O. Characterization and engineering of a plastic-degrading aromatic polyesterase. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115* (19), 201718804.
- (24) Kaplan, D. L.; Hartenstein, R.; Sutter, J. Biodegradation of polystyrene, poly(methyl methacrylate), and phenol formaldehyde. *Appl. Environ. Microbiol.* **1979**, *38* (3), 551–553.
- (25) Gautam, R.; Bassi, A. S.; Yanful, E. K. A review of biodegradation of synthetic plastic and foams. *Appl. Biochem. Biotechnol.* **2007**, *141* (1), 85–108.
- (26) Nakamiya, K.; Sakasita, G.; Ooi, T.; Kinoshita, S. Enzymatic degradation of polystyrene by hydroquinone peroxidase of Azotobacter beijerinckii HM121. *J. Ferment. Bioeng.* **1997**, *84* (84), 480–482.
- (27) Tian, L.; Kolvenbach, B.; Corvini, N.; Wang, S.; Tavares, N.; Wang, L.; Ma, Y.; Scheu, S.; Corvini, F. X.; Rong, J. Mineralisation of 14 C-labelled polystyrene plastics by *Penicillium variabile* after ozonation pre-treatment. *New Biotechnol.* **2017**, *38*, 101–105.
- (28) Mor, R.; Sivan, A. Biofilm formation and partial biodegradation of polystyrene by the actinomycete *Rhodococcus ruber*. *Biodegradation* **2008**, *19* (6), 851–858.
- (29) Shah, A. A.; Hasan, F.; Hameed, A.; Ahmed, S. Biological degradation of plastics: a comprehensive review. *Biotechnol. Adv.* **2008**, *26* (3), 246–65.
- (30) Sivan, A. New perspectives in plastic biodegradation. *Curr. Opin. Biotechnol.* **2011**, *22* (3), 422–6.
- (31) Krueger, M. C.; Harms, H.; Schlosser, D. Prospects for microbiological solutions to environmental pollution with plastics. *Appl. Microbiol. Biotechnol.* **2015**, *99* (21), 8857–8874.
- (32) Yang, Y.; Yang, J.; Wu, W. M.; Zhao, J.; Song, Y.; Gao, L.; Yang, R.; Jiang, L. Biodegradation and Mineralization of Polystyrene by Plastic-Eating Mealworms: Part 2. Role of Gut Microorganisms. *Environ. Sci. Technol.* **2015**, *49* (20), 12087–93.
- (33) Yang, S. S.; Wu, W. M.; Brandon, A. M.; Fan, H. Q.; Receveur, J. P.; Li, Y.; Wang, Z. Y.; Fan, R.; McClellan, R. L.; Gao, S. H.; Ning, D.; Phillips, D. H.; Peng, B. Y.; Wang, H.; Cai, S. Y.; Li, P.; Cai, W. W.; Ding, L. Y.; Yang, J.; Zheng, M.; Ren, J.; Zhang, Y. L.; Gao, J.; Xing, D.; Ren, N. Q.; Waymouth, R. M.; Zhou, J.; Tao, H. C.; Picard, C. J.; Benbow, M. E.; Criddle, C. S. Ubiquity of polystyrene digestion and biodegradation within yellow mealworms, larvae of *Tenebrio*

- molitor Linnaeus (Coleoptera: Tenebrionidae). *Chemosphere* **2018**, *212*, 262–271.
- (34) Yang, S. S.; Brandon, A. M.; Andrew Flanagan, J. C.; Yang, J.; Ning, D.; Cai, S. Y.; Fan, H. Q.; Wang, Z. Y.; Ren, J.; Benbow, E.; Ren, N. Q.; Waymouth, R. M.; Zhou, J.; Criddle, C. S.; Wu, W. M. Biodegradation of polystyrene wastes in yellow mealworms (larvae of *Tenebrio molitor* Linnaeus): Factors affecting biodegradation rates and the ability of polystyrene-fed larvae to complete their life cycle. *Chemosphere* **2018**, *191*, 979–989.
- (35) Brandon, A. M.; Gao, S. H.; Tian, R.; Ning, D.; Yang, S. S.; Zhou, J.; Wu, W. M.; Criddle, C. S. Biodegradation of Polyethylene and Plastic Mixtures in Mealworms (Larvae of *Tenebrio molitor*) and Effects on the Gut Microbiome. *Environ. Sci. Technol.* **2018**, *52* (11), 6526–6533.
- (36) Gerhardt, P.; Lindgren, D. Penetration of packaging films: Film materials used for food packaging tested for resistance to some common stored-product insects. *J. Econ. Entomol.* **1954**, *47* (2), 282–287.
- (37) Cline, L. D. Penetration of Seven Common Flexible Packaging Materials by Larvae and Adults of Eleven Species of Stored-Product Insects. *J. Econ. Entomol.* **1978**, *71* (5), 726–729.
- (38) Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., 2017. Vegan: Community Ecology Package. R Package Version 2.4–2. 2017. <https://CRAN.R-project.org/package=vegan>.
- (39) Wikipedia. Information for *Tenebrio*. <https://en.wikipedia.org/wiki/Tenebrio>.
- (40) National Center for Biotechnology Information. Information for *Tenebrio*. <https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=7066>.
- (41) Calmont, B.; Soldati, F. Ecologie et biologie de *Tenebrio opacus* Duftschmid, 1812 Distribution et détermination des espèces françaises du genre *Tenebrio* Linnaeus, 1758 (Coleoptera, Tenebrionidae). *R.A.R.E.T.* **2008**, *27* (3), 81–87.
- (42) Robinson, W. H. *Urban insects and arachnids: a handbook of urban entomology*; Cambridge University Press: 2005.
- (43) Canadian Grain Commission. Information for *Tenebrio obscurus* Fabricius 1792. <https://www.grainscanada.gc.ca/storage-entrepose/sip-irs/dm-to-eng.htm>.
- (44) Wikipedia. Information for *Zophobas morio*. [https://en.wikipedia.org/wiki/Zophobas\\_morio](https://en.wikipedia.org/wiki/Zophobas_morio).
- (45) Baidu Encyclopedia (in Chinese). Information for *Tenebrio obesus*. <https://baike.baidu.com/item/%E9%BB%91%E7%B2%89%E8%99%AB>.
- (46) Zhang, J.; Kober, K.; Flouri, T.; Stamatakis, A. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* **2014**, *30* (5), 614.
- (47) Edgar, R. C. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **2013**, *10* (10), 996.
- (48) Singh, B.; Sharma, N. Mechanistic implications of plastic degradation. *Polym. Degrad. Stab.* **2008**, *93* (3), 561–584.
- (49) Ho, B. T.; Roberts, T. K.; Lucas, S. An overview on biodegradation of polystyrene and modified polystyrene: the microbial approach. *Crit. Rev. Biotechnol.* **2018**, *38* (2), 308.
- (50) Lemos, L. N.; Fulthorpe, R. R.; Triplett, E. W.; Roesch, L. F. W. Rethinking microbial diversity analysis in the high throughput sequencing era. *J. Microbiol. Methods* **2011**, *86* (1), 42–51.
- (51) Yang, J.; Yang, Y.; Wu, W. M.; Zhao, J.; Jiang, L. Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms. *Environ. Sci. Technol.* **2014**, *48* (23), 13776–84.
- (52) Wang, Y.; Zhang, Y. Investigation of Gut-Associated Bacteria in *Tenebrio molitor* (Coleoptera: Tenebrionidae) Larvae Using Culture-Dependent and DGGE Methods. *Ann. Entomol. Soc. Am.* **2015**, *108* (5), 941–949.
- (53) Engel, P.; Moran, N. A. The gut microbiota of insects – diversity in structure and function. *FEMS Microbiol. Rev.* **2013**, *37* (5), 699–735.
- (54) Wang, L.; Lilburn, M.; Yu, Z. Intestinal Microbiota of Broiler Chickens As Affected by Litter Management Regimens. *Front. Microbiol.* **2016**, *7*, 593.
- (55) Chen, M.; Zhang, X.; Wang, Z.; Liu, M.; Wang, L.; Wu, Z. Impacts of quaternary ammonium compounds on membrane bioreactor performance: Acute and chronic responses of microorganisms. *Water Res.* **2018**, *134*, 153–161.
- (56) *Tenebrio molitor* distribution worldwide (2). [http://eol.org/data\\_objects/21466806](http://eol.org/data_objects/21466806).
- (57) *Tenebrio obscurus* distribution worldwide (1). [http://eol.org/data\\_objects/21466807](http://eol.org/data_objects/21466807).