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Distinct assembly processes of intestinal and non-intestinal microbes of bark beetles from clues of metagenomic insights

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Ips **(Curculionidae: Scolytinae) bark beetles (BBs) are ecologically and economically devastating coniferous pests in the Northern Hemisphere. Although the microbial diversity associated with these beetles has been well studied, mechanisms of community assembly and the functional roles of key microbes remain poorly understood. This study investigates the microbial community structures and functions in both intestinal and non-intestinal environments of five *Ips* BBs using a metagenomic approach. The findings reveal similar microbial community compositions, though the α-diversity of dominant taxa differs between intestinal and non-intestinal environments due to the variability in bark beetle species, host trees, and habitats. Intestinal microbial communities are predominantly shaped homogenizing dispersal (HD) and undominated processes (UP), whereas non-intestinal microbial communities are primarily driven by heterogeneous selection (HS). Functional analysis shows that genes and enzymes associated with steroid biosynthesis and oxidative phosphorylation are primarily found in non-intestinal fungal symbionts *Ogataea*, *Wickerhamomyce*, *Ophiostoma*, and *Ceratocystis* of *Ips* species. Genes and enzymes involved in degrading terpenoids, phenolic compounds, and polysaccharides are predominately found in the intestinal *Acinetobacter*, *Erwinia*, and *Serratia*. This study provides valuable and in-depth insights into the symbiotic relationships between *Ips* BBs and their microbial partners, enhancing our understanding of insect-microbe coevolution and suggesting new strategies for pest management.**

Keywords Bark beetles, Core taxa, Unigenes, Complex compounds, Stochastic process, Deterministic process

Symbiotic microorganisms significantly influence the physiology, ecology and evolution of their hosts[1,2]. These microbes which included fungi, bacteria, viruses, archaea, protists, and helminths inhabit both intestinal and non-intestinal samples[1,3–6]. High-throughput sequencing (HTS) has been widely used to analyze these microbiomes, uncovering their distribution characteristics and microbial community diversity[7–10]. The intestine, as a specific ecological habitat for food intake, storage and digestion in insects, is inhabited by a rich diversity of microorganisms[11,12]. Microbial community composition varies across insect species. For example, *Snodgrassella alvi* Kwong and Moran, *Gilliamella apicola* Kwong and Moran, *Lactobacillus*, and *Bifidobacterium* core intestinal microbes in *Apis mellifera* Linnaeus[13]; *Enterobacter*, *Serratia*, *Acinetobacter*, and *Micrococcus* are common in *Curculio chinensis* Chevrolat[14]. In bark beetles, core members of the intestinal microbes include yeasts, ophiostomatoid fungi, *Pantoea*, *Pseudomonas*, and *Rahnella*[1,15]. These intestinal microbes community structures are linked to host diets (e.g., carnivorous versus herbivorous beetles) and the environment (e.g., in the wild versus indoor breeding)[16,17]. Intestinal microbes play various roles in maintaining host insect health[18], and protecting the host from pathogens[13]. It can provide nutrients to host by degrading complex compounds such as lignocellulose, cellulose, and pectin[19,20]. These microbes can also detoxify plant defense compounds (terpenes, alkaloids, and phenols), providing nutrients[21–23].

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Non-intestinal tissues such as the cuticle, oral cavity, antennal segments, and glandular reservoirs serve as important microbial habitats. The cuticle, in particular, is a major structure for inhabiting microorganisms[1,3]. For example, BBs carry various fungi on their exoskeleton or in specialized structures like mycetangia[24]. Studies have shown that some common crucial microbial taxa are harbored in the non-intestinal samples[25,26]. These fungal symbionts synthesize sterols, fatty acids and amino acids critical for the beetle's growth, development, reproduction, and colonization of BBs[27–29]. Non-intestinal fungal symbionts can also synthesize pheromones to assist bark beetles in accomplishing colonization and population establishment[30]. Additionally, fungal pathogens harbored in non-intestinal tissues can infect the tunnel[31].

Ips (Coleoptera: Scolytinae) BBs primarily feed on the phloem of coniferous trees like *Picea*, *Pinus* and *Larix*, causing catastrophic ecological and economical damage to forestry in the Northern Hemisphere[25,32,33]. For example, *Ips nitidus* Eggers damages *Picea crassifolia* forests on the Qinghai-Tibet Plateau[32,34]. Hauser's engraver beetle (*Ips hauseri* Reitter) mainly distributes in mountainous areas of Central Asia and has an important influence on *Picea schrenkiana*, *Picea obovata* and *Pinus sylvestris*[35,36]. *Ips chinensis* Kurentzov and Kononov mainly distributes in southern and northern China, which can cause substantial damage to pine forests, such as *Pinus koraiensis*[37]. And *I. typographus* Linnaeus and *I. subelongatus* Motschulsky have disastrously ecological and economic loss for coniferous forests with the assistance of microbial symbionts[38–41]. BBs and microorganisms, serving as a classic symbiosis model, play a pivotal role in augmenting the adaptability to environment, evolutionary strategies and maintaining specific ecological functions[24,28]. Although microbial composition and diversity are studied in the intestines or non-intestines of multiple BBs, it lacks direct comparison for the microbial community structure in the intestinal and non-intestinal samples of *Ips* bark beetle species in China.

Additionally, interactions can regulate microbial community structures and multiple functionalities to affect host insects[42,43]. Bacterial associates can mediate the fungal symbionts *Leptographium procerum* (W.B. Kendr.) M.J. Wingf. and *Ophiostoma minus* (Hedgc.) Syd. & P. Syd. to consume D-pinitol before consuming D-glucose in red turpentine beetle larvae[44]. Studies elucidated that insects can survive in specific environments owing to the driver in microbial community assembly, such as immigration, weak selection, and drift. The community assembly processes are driven by deterministic and stochastic processes[45]. The former includes environmental and biotic filtering[46], the latter includes random birth, death, and dispersal events[47]. Stochasticity is likely to govern temporal and spatial variation in species richness[48]. For example, dispersal limitation (DL) and drift governed intestinal microbial community assemblies in flies, beetles, and honeybees[42,49]. After establishing microbial community, deterministic processes may become important when spatial variation leads to enhanced environmental selection. Microbial community assembly is driven by stochastic to deterministic processes[48]. However, relative importance of symbiotic microbial community assemblies for BBs are poor, hindering our cognition of symbiosis mechanisms between BBs and their partners. So, exploring the dominant driving forces of microbial community assembly is crucial for understanding the mechanisms underlying biodiversity and maintaining ecological system function[48].

In this study, metagenomics was used to investigate the microbial community structure and functional traits in the intestines and non-intestines for five representative *Ips* BBs in China. The objectives were to: (1) compare fungal and bacterial community composition in the intestines and non-intestines; (2) determine microbial interaction and community assembly mechanism in the intestinal and non-intestinal samples; and (3) explore bacterial and fungal functional genes and enzymes in the intestinal and non-intestinal samples, which are related to sterol synthesis and the degradation of complex compounds. This is the first study to compare intestinal and non-intestinal microbial community structures, revealing distinct driving forces that shape the microbial community assemblies in BBs. Our findings enhance the understanding of insect-microbe coevolution, offering insights into mutualistic mechanisms and novel strategies for pest and disease control.

Materials and methods

Sample collection and processing

Five *Ips* BBs were collected from five locations from July 2020 to September 2022 (Fig. 1a; Table 1). Sterile forceps were used to move emerged adults of the second generation from different galleries (Fig. 1b–h), and all samples were stored in 2 mL sterile Eppendorf tubes without surface sterilization. Identification was based on geographical distribution, feeding trees and morphological characteristics[38,50]. A total of 210 emerged adults of each *Ips* bark beetle were randomly collected for the same log and divided into three groups. Intestines were extracted according to the methods described by Delalibera et al.[51] and Morales-Jiménez et al.[52]. The remaining tissues were used as non-intestinal samples, resulting in three intestinal and three non-intestinal samples per bark beetle species. A total of 1,050 adults (30 samples) were sampled and stored in a −80 ℃ laboratory ultra-low temperature freezer. The liquid nitrogen quick-freezing method was used to prevent contamination and ensure consistency with the microbial communities in the field environment.

DNA extraction, sequencing, assembly, and annotation

Samples were mixed before DNA extraction, with 500 mg per sample used for total DNA extraction following the CTAB method. DNA quality was evaluated using the Qubit™ dsDNA HS Assay Kit (Invitrogen, Qubit 3.0) and 1% agarose gel electrophoresis. DNA libraries were constructed using the VAHTSR Universal Plus DNA Library Prep Kit for Illumina. Sequencing was performed by Beijing Biomarker Biotechnological Company using Illumina NovaSeq6000 (San Diego: NovaSeq 6000 S4 Reagent Kit). Fastp software was used for quality control and filtering of raw reads to acquire high-quality sequencing data[53]. Bowtie 2[54] was used to eliminate reads originating from the host, and the aligned reference genomes were *Ips nitidus* (GenBank accession number: GCA_018691245.2) and *I. typographus* (GenBank accession number: GCA_016097725.1). Metagenomes were assembled using MEGAHIT[55] version 1.1.2 software (https://github.com/voutcn/megahit) after filtering contigs less than 300 bp. Assembly results were evaluated using QUAST[56] and genomic coded regions were identified

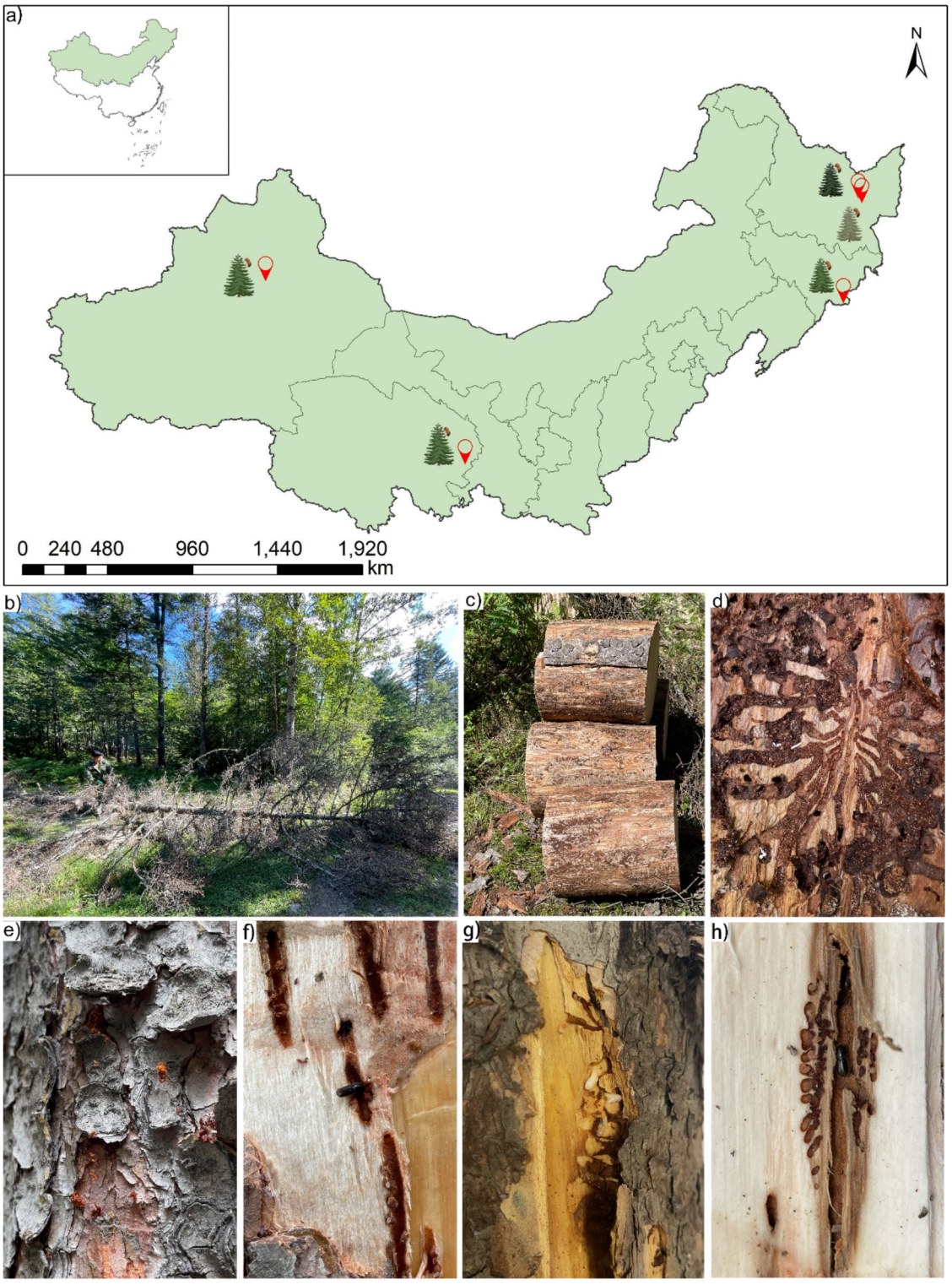

Fig. 1. Sampling locations, typical habitats, and selected life stage for *Ips* BBs. (**a**) Sampling sites in five locations of China, and the map using QGIS 3.34.13 (https://qgis.org/). (**b**–**d**) Symptoms and galleries on spruce infested by *I. typographus*; (**e**–**h**). Frass, eggs, larvae, pupae, and adults of *I. hauseri* in spruce galleries.

in MetaGeneMark version 3.26 software[57]. Gene prediction was performed with default parameters, and gene redundancy with 95% similarity and 90% coverage thresholds were determined using MMseq2 version 11-e1a1c[58]. Finally, 30 metagenomic datasets were uploaded to NCBI to obtain bioproject ID, biosample accessions and Sequence Read Archive (SRA) (Supplementary Table S1).

Species name	Sample ID	Host tree	Latitude longitude	Elevation/m	Location
Ips subelongatus	ISC	*Larix olgensis*	46.4235 N 130.67387 E	250	HMJG
	IS				
Ips chinensis	ICC	*Pinus koraiensis*	46.191056 N 131.284223 E	420	HXY
	IC				
Ips typographus	ITC	*Picea jezoensis* var. *komarovii*	41.482483 N 127.831204 E	761	JTD
	IT				
Ips hauseri	IHC	*Picea schrenkiana* var. *tianschanica*	43.38847 N 87.394593 E	1925	XTS
	IH				
Ips nitidus	INC	*Picea crassifolia*	35.271778 N 101.924683 E	3016	QMX
	IN				

Table 1. Sample collection information of five *Ips* BBs from different areas and host trees. ISC, ICC, ITC, IHC, INC representing intestinal samples; IS, IC, IT, IH, IN representing non-intestinal samples. *Ips* BBs collection from three host trees (larch, pine and spruce) across locations in Mengjiagang (HMJG) and Xiangyang (HXY) forest farm in Heilongjiang province; Thirteen Daogou (JTD) forest farm in Jilin province, the Tianshan grand canyon (XTS) in Xinjiang Urumqi; and Maixiu (QMX) nature reserve in Qinghai province, respectively.

A non-redundant protein sequence database (NR) was used to annotate the taxonomic identification of the symbiotic microbial communities for five *Ips* BBs. Subsequently, functional genes and enzymes were annotated using the KEGG database[59] (with default parameters e value ≤ 1e − 5) and Carbohydrate-active enzymes (CAZy) database. The highest similarity and annotation information of genes were matched to the corresponding KEGG orthologs (KO).

Community composition, alpha- and beta- diversity analysis

Rarefaction curves were used to show the sequencing depth of species and genes for all samples before analyzing microbial community structure. The microbial community compositions of intestinal (ISC, ICC, ITC, IHC and INC) and non-intestinal (IS, IC, IT, IH and IN) samples were analyzed at the phylum, order and genus levels. The relative abundances of the dominant microbes were compared across *Ips* BBs at different host trees (larch, pine, and spruce). Alpha-diversity (α-diversity) at the genus level were performed using student's t-test. Non-metric multidimensional scaling (NMDS) was calculated with Bray-Curtis' dissimilarities based on the PERMANOVA test to determine the difference between species and samples. The analyses were performed online using BMKCloud (https://www.biocloud.net/) platforms.

Co-occurrence network analysis

Co-occurrence networks were construct in R version 4.2.2 with "igraph", "Hmisc", "ggplot2", "ggpubr", and "ggsignif" packages to confirm important interrelationships between intestinal and non-intestinal microbial communities for five *Ips* BBs. Microbes with an incidence rate below 50% were filtered to obtain clear network interrelationships. Correlations were regarded as statistically significant with $r > 0.7$ and $p < 0.05$. Spearman correlation index and Benjamini-Hochberg (FDR-BH) method were applied for statistical testing. The network was exhibited in Gephi version 0.10.1 software[60] and microbial interactions were assessed using parameters such as average clustering coefficient, average degree, average path length, network diameter, map density, and average weighted degree.

Community assembly analysis of core taxa

All gene sequences for core taxa were aligned with ClustalW1.6[61], and ML trees were constructed with 5000 bootstrap replicates using IQ-tree in PhyloSuite version 1.2.3[62]. Core taxa included ophiostomatoid fungi (*Ophiostoma*, *Leptographium*, *Grosmannia*, *Esteya*, *Sporothrix*, Ophiostomataceae unclassified, *Endoconidiophora*, *Ceratocystis*, *Thielaviopsis*, and Ceratocystidaceae unclassified), yeast (Schizosaccharomycetales and Saccharomycetales), *Enterobacter*, *Acinetobacter*, *Serratia*, *Erwinia*, *Mycobacterium*, and *Mycolicibacterium*). These microbes play crucial roles in community composition, nutritional provision, and complex compounds degradation[63,64]. Weighted β-nearest taxon index (βNTI) and β-Raup-Crick metric (RC) dissimilarity were calculated using the "picante" package in "R" to evaluate microbial community assembly processes. The βNTI value > 2 represented heterogeneous selection (HS), while βNTI < -2 indicated homogeneous selection (HOS), both representing deterministic processes. |βNTI| was less than 2, the stochasticity was deemed the main driving force. The RC value greater than 0.95 suggested that community assembly was dominated by dispersal limitation (DL). RC value smaller than − 0.95 indicated that homogenizing dispersal (HD) was the primary driving force. Additionally, |RC| ≤ 0.95 indicated that undominated processes (UP) (drift, diversification, weak selection and weak dispersal) governed the community assembly[45,65].

Functional genes and enzymes analysis for microbial communities

The number of fungal and bacterial unigenes was compared in the KEGG enrichment (level 2) analysis. The difference of gene relative abundance was compared among all samples using the complete hierarchical clustering method and Euclidean distance calculation in the KEGG pathway (level 3). Genes and enzymes linked to steroid biosynthesis, polysaccharides, terpenoids and phenolic compounds degradation were annotated.

Phenolic-related products were annotated in the KEGG and CAZy databases. These analyses were mainly performed online on GenesCloud (https://www.genescloud.cn) or OmicStudio Cloud (https://www.omicstudio.cn) platforms.

Results

Sequencing of intestinal and non-intestinal samples

Rarefaction curves based on species and genes were close to asymptote in all samples, indicating sufficient sequencing depth for both intestinal and non-intestinal samples (Supplementary Fig. S1a–d). Following filtration, the number of assembled contigs per species ranged from 155,572 to 342,753, with the base largest length of contigs between 9,314 and 52,264. The N50 ranged from 596 to 1,558 bp, the GC content from 32.54 to 35.90%, and the proportion of mapped contigs (assembled contigs / sequenced reads) from 81.94% to 99.65%. The number of predicted functional genes ranged from 48,044 to 138,187, with maximum base length between 3,444 and 9,900 bp (Table 2). In total, 1,106,180 non-redundant genes were obtained, with an average base length of 252 bp and a maximum largest length of 9,900 bp. A total of 113,332 metagenes were assigned to 6,116 KOs, representing 996 fungi and 5,351 bacteria.

Comparison of intestinal and non-intestinal microbial community composition

Results showed a higher relative abundance of Microsporidia in the intestinal samples compared to non-intestinal samples. Non-intestinal samples exhibited slightly higher relative abundances of Ascomycota, Mucoromycota, unclassified group, and Basidiomycota were slightly higher than those of the intestinal samples (Fig. 2a). At the order level, Erysiphales, Mucorales, Saccharomycetales and Pucciniales were more abundant in non-intestinal samples than in the intestinal samples (Fig. 2b). At the genus level, non-intestinal *Erysiphe* and *Puccinia* showed slightly higher relative abundances than those in the intestinal samples (Fig. 2c). The relative abundances of Saccharomycetales and Ophiostomatoid fungi were slightly lower in the intestines than those of non-intestines for *I. subelongatus* and *I. chinensis*. Conversely, these two groups associated with spruce-feeding

Group	Sample ID	No. of contigs (assembly)	Largest length of contigs (bp)	N50 (bp)	No. of genes	Max base length of predicted genes	GC content (%)	Mapped (%)
Intestine	ISC1	216,954	17,329	1416	83,326	4809	33.18	98.44
	ISC2	227,459	20,895	1444	85,636	4593	33.09	98.85
	ISC3	223,902	17,965	1482	86,683	4383	33.17	98.63
	ICC1	254,666	23,858	1264	92,947	8418	32.64	99.10
	ICC2	238,393	51,745	1268	87,580	8217	32.73	99.15
	ICC3	239,411	28,051	1275	85,988	8232	32.56	99.49
	ITC1	248,407	23,135	814	84,228	5430	33.98	96.19
	ITC2	244,731	22,444	825	84,888	5439	34.14	96.90
	ITC3	241,505	18,554	854	87,099	4812	34.22	96.67
	IHC1	230,555	40,709	1509	91,842	7548	34.11	98.85
	IHC2	218,409	52,264	1558	91,416	8112	34.23	98.44
	IHC3	246,950	34,415	1432	102,007	9141	34.37	98.16
	INC1	187,796	33,686	596	61,786	3927	34.72	81.94
	INC2	155,572	9314	639	48,044	3897	33.49	88.56
	INC3	203,563	12,665	603	64,510	3444	34.55	87.54
Non-intestine	IS1	243,811	23,268	1303	95,682	4962	33.58	96.95
	IS2	342,753	21,042	1173	138,187	5298	34.19	96.68
	IS3	262,740	18,329	1257	116,160	4551	34.12	96.08
	IC1	254,388	23,769	1266	89,664	9900	32.54	98.95
	IC2	272,045	51,774	1047	90,373	8961	32.74	98.93
	IC3	235,870	21,892	1282	85,291	8385	32.59	99.65
	IT1	274,779	22,817	826	101,777	5430	34.87	94.56
	IT2	257,349	26,348	821	91,321	6642	34.79	94.60
	IT3	292,536	42,235	818	107,081	7347	35.18	94.98
	IH1	218,300	28,330	1496	91,741	5940	34.34	98.23
	IH2	188,305	22,435	1530	81,850	7722	34.35	98.6
	IH3	195,938	28,244	1479	86,706	8769	34.59	97.81
	IN1	160,926	41,175	1163	88,446	8724	35.90	93.02
	IN2	170,986	11,953	632	50,498	4365	34.14	87.46
	IN3	185,973	49,455	1016	95,173	9552	35.88	92.23

Table 2. Samples sequencing information of microbes in the intestines and non-intestines for five *Ips* BBs. Mapped representing ratio of assembly contigs and sequenced reads.

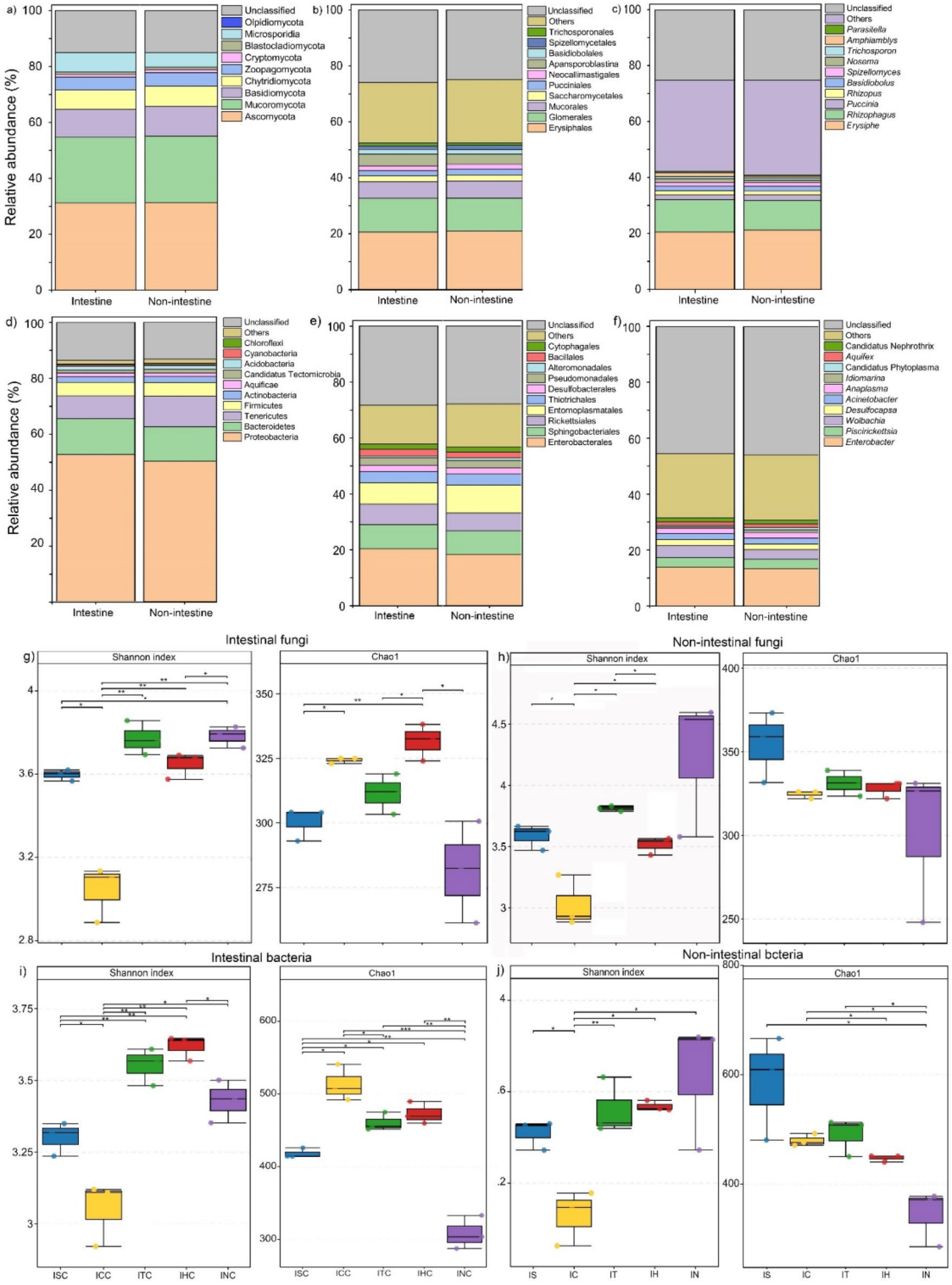

Fig. 2. Microbial community structure in the intestines and non-intestines of five *Ips* BBs at different taxonomic levels. (**a**–**c**) Fungal community composition and (**d**–**f**) bacterial community composition at the phylum, order and genus. (**g**) α-diversity (Shannon index and Chao1 index) in the intestinal fungal symbionts of every *Ips* bark beetle based on student's t-test (*$p < 0.05$, **$0.01 < p < 0.05$, ***$p < 0.01$) at the genus level; (**h**) α-diversity in the non-intestinal fungal symbionts; (**i**) α-diversity in the intestinal bacterial associates; (**j**) α-diversity in the non-intestinal bacterial associates.

beetles were slightly more abundant in the intestinal samples. Additionally, *I. nitidus* non-intestinal samples showed the higher relative abundance of Saccharomycetales associated with non-intestinal samples from other *Ips* BBs (Supplementary Table S2).

Intestinal samples showed higher relative abundances of Proteobacteria, Bacteroidetes, Firmicutes, Aquificae and Acidobacteria compared to non-intestinal samples (Fig. 2d). At the order level, Enterobacterales, Sphingobacteriales, Rickettsiales, Pseudomonadales, and Bacillales were more prevalent in the intestinal samples than in non-intestinal ones (Fig. 2e). *Enterobacter* had a higher relative abundance in the intestines of *I. subelongatus*, *I. typographus*, *I. hauseri* and *I. nitidus*. *Piscirickettsia* and *Wolbachia* were more abundant in the intestines of *I. chinensis* and *I. nitidus* (Fig. 2f). *Acinetobacter* and *Escherichia* were more abundant in the intestines than in the non-intestines of *I. hauseri* and *I. nitidus*. *Serratia* and *Erwinia* had higher abundance in the intestinal samples of *I. chinensis*, *I. typographus* and *I. nitidus*. *Klebsiella*, *Microbacterium*, *Rickettsia* and *Pseudomonas* exhibited higher relative abundances in the intestinal tracts of *I. chinensis*, *I. hauseri* and *I. nitidus* compared to the non-intestinal tissues (Supplementary Table S2).

The microbial community diversity was compared between the intestinal and non-intestinal samples of five *Ips* BBs (Fig. 2g–j). The Shannon index showed highest species diversity in the intestines of *I. typographus* and *I. hauseri* for both fungi (ITC: 3.77, IHC: 3.65) and bacteria (ITC: 3.55, IHC:3.62) (Fig. 2g). In non-intestinal samples, the highest diversity was observed in *I. nitidus* and *I. typographus* for fungi (IN: 4.24, IT: 3.81) and bacteria (IN: 3.67, IT: 3.52) compared to other three *Ips* BBs (Fig. 2h, j). Chao1 index indicated greater species richness of associated fungi (IHC: 331.59, ICC: 324.33) and bacteria (IHC: 472.89 and ICC: 513.38) in the intestinal tissues of *I. hauseri* and *I. chinensis* (Fig. 2g, i). It had significantly greater species richness for fungal (IS: 354.67, IT: 331.26) and bacterial (IS: 585.39, IT: 490.30) communities in the non-intestinal samples of *I. subelongatus* and *I. typographus* than in those of other three *Ips* BBs (Fig. 2h, j). While fungal diversity associated with *I. nitidus* non-intestines showed no clear differences among the other four *Ips* BBs, intestinal fungal and bacterial diversity were notably different from those of *I. subelongatus*, *I. hauseri*, and *I. chinensis*. Non-metric multidimensional scaling (NMDS) showed remarkable differences (PERMANOVA $R^2 = 0.8$, $p = 0.001$) in the fungal (Supplementary Fig. S1e) and bacterial communities (Supplementary Fig. S1f) associated with intestinal and non-intestinal samples of five *Ips* BBs.

In all cases, although there was similar community composition of symbiotic fungi and bacteria in the intestinal and non-intestinal samples, the relative abundances varied with different taxa from two type samples of *Ips* BBs. The relative abundances of intestinal fungi were slightly lower than those of non-intestinal samples. In contrast, the relative abundances of intestinal bacteria were slightly higher than those of the non-intestine. Saccharomycetales and Enterobacterales exhibited the higher diversity and relative abundances in the intestines and non-intestines of different *Ips* BBs. And intestinal and non-intestinal fungi and bacteria with *I. typographus* had greater species richness and diversity. Microbial community structures were linked to different dietary trees (larch, spruce and pine), host beetles, specific habitats and sampling sites.

Microbial interaction and community assembly processes

Network analysis showed 100% positive connections among the intestinal (fungi: 45.49%, bacteria: 54.51%) (Fig. 3a) and non-intestinal microbes (fungi: 46.48%, bacteria: 53.52%) (Fig. 3b), suggesting the mutualistic interactions between fungi and bacteria associated with five *Ips* BBs. The results showed that the connectivity of intestinal microbes was tighter than non-intestinal ones. The average degree of intestinal microbes (135.166) was significantly more than twice as large as non-intestinal ones (65.202). Moreover, the number of connections for intestinal microbes (83,938) was remarkably higher than non-intestines (43,066) (Table 3). Additionally, there were significant differences between the intestinal and non-intestinal samples ($p = 2.22e\text{-}16 < 0.01$) (Fig. 3c). In the intestinal microbial network analysis, dominant genera with high interactions included unclassified bacteria (1.69%), *Aspergillus* (1.29%), *Bacillus* (1.05%), unclassified Gammaproteobacteria (0.97%), unclassified Bacteroidetes (0.81%), *Enterobacter* (0.72%), *Paenibacillus* (0.72%), *Pseudomonas* (0.64%), unclassified Alphaproteobacteria (0.56%), unclassified Deltaproteobacteria (0.40%), *Streptomyces* (0.32%), *Acinetobacter* (0.24%), *Pantoea* (0.24%), *Clostridium* (0.24%), *Chryseobacterium* (0.16%), *Microbacterium* (0.16%), *Serratia* (0.08%), *Mycolicibacterium* (0.08%) and others (89.61%). These dominant genera were harbored in the non-intestines, except for *Pantoea* and *Serratia*, which may be notably more significant in the intestinal environment.

The results showed that HD was the primary driving force of all core microbial community assemblies except *Mycobacterium* in the intestines of *I. subelongatus*. However, the non-intestinal microbial community assembly of *I. subelongatus* was more inclined to be driven by HS. Stochastic process (HD, DL and UD) drove the core microbial community assemblies in the intestinal and non-intestinal samples of *I. chinensis*. HD and UD emerged as dominant drivers governing intestinal microbial community assemblies of *I. typographus* and *I. nitidus*. HOS and HS in the non-intestines of *I. typographus* and HS in the non-intestines of *I. nitidus* had more relatively greater contributions to the microbial assemblies. HD was the main governing force in the intestinal and non-intestinal samples of *I. hauseri* (Fig. 3d–g; Table 4; Supplementary Table S3). In sum, stochastic processes (HD and UD) appeared to be entirely dominant driving forces in the intestinal microbial community assemblies of five *Ips* BBs. However, non-intestinal core microbial community assemblies may be preferentially governed by deterministic processes (HS).

Functional genes annotation for fungal and bacterial communities

In total, 20,459, 25,886 fungal unigenes were annotated in the intestinal and non-intestinal samples (Fig. 4a, b). For bacteria unigenes, 25,550 and 30,677 were annotated in the intestinal and non-intestinal samples (Fig. 4c, d). These genes were subjected to KEGG enrichment analysis and categorized into 22 KEGG pathway level 2, including cellular processes (4 pathways), environmental information processing (2 pathways), genetic information processing (4 pathways) and metabolism (12 pathways). The results revealed that the number of

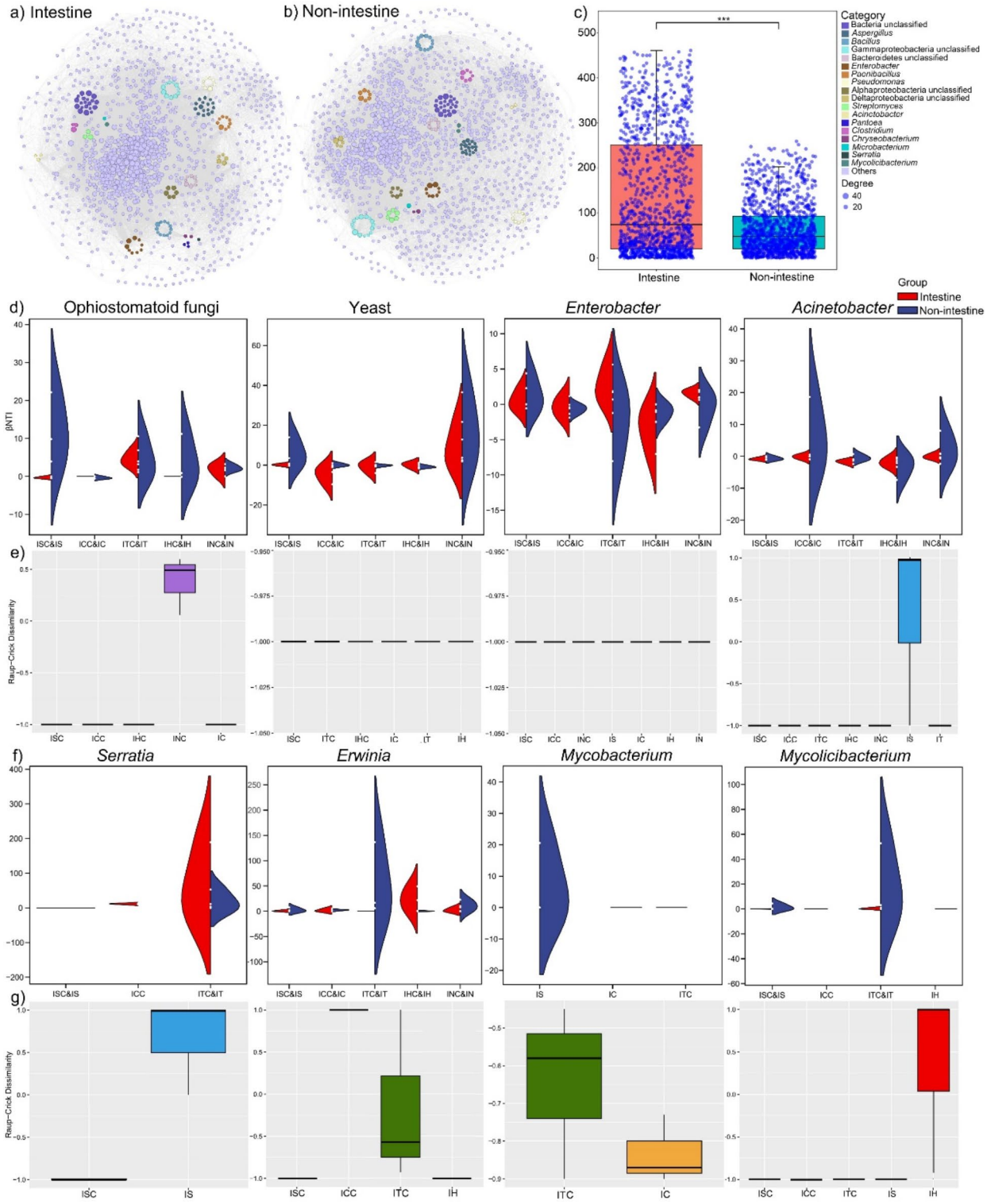

Fig. 3. Co-occurrence networks and microbial assembly processes of intestinal and non-intestinal fungi and bacteria associated with different *Ips* BBs. Co-occurrence network analysis of intestinal (**a**) and non-intestinal (**b**) fungal and bacterial communities with statistically significant Spearman correlations ($r > 0.7$, $p < 0.05$), and the node size representing relative abundances of different species. The higher the average degree, the higher the network complexity. (**c**) Significant difference of network in the intestinal and non-intestinal tissues for five *Ips* BBs. (**d**–**g**) Community assemblies of crucial microbiomes. "+" representing |βNTI| > 2, deterministic process; "-" representing |βNTI| < 2, stochastic process; "HS" and "HOS" representing heterogeneous and homogeneous selection of deterministic process; "HD" and "DL" representing homogenizing dispersal and dispersal limitation of stochastic process; βNTI > 2 representing HS, βNTI < −2 representing HOS; RC < -0.95 representing HD, RC > 0.95 representing DL, |RC| ≤ 0.95 meaning UD.

L	*N*	C	PC	M	ACC	APL	ND	AD	MD	AWD
Intestine	1242	83,938	83,938(100%)	0.283	0.603	2.977	11	135.166	0.109	107.324
Non-intestine	1321	43,066	43,066(100%)	0.498	0.511	3.154	8	65.202	0.049	50.809

Table 3. The parameters of co-occurrence networks for fungi and bacteria associated with *Ips* BBs in the intestinal and non-intestinal samples. *L* location; *N* node; *C* connection; *PC* positive connection; *M* modularity; *ACC* average clustering coefficient; *APL* average path length; *ND* network diameter; *AD* average degree; *MD* map density; *AWD* average weighting degree.

Group	ISC	ICC	ITC	IHC	INC	IS	IC	IT	IH	IN
Ophiostomatoid fungi	−HD	−HD	+HS	−HD	−UD	+HS	−HD	+HS	+HS	+HS
Yeast	−HD	+HOS	−HD	−HD	+HS	+HS	−HD	−HD	−HD	+HS
Enterobacter	−HD	−HD	+HS	+HOS	−HD	−HD	−HD	+HOS	−HD	−HD
Acinetobacter	−HD	−HD	−HD	−HD	−UD	−UD	+HS	−HD	+HS	+HS
Serratia	−HD	+HS	+HS			−UD		+HS		
Erwinia	−HD	−DL	−UD	+HS	+HS	+HS	+HS	+HS	−HD	+HS
Mycobacterium			−UD			+HS	−UD			
Mycolicibacterium	−HD	−HD	−HD			−UD			−HD	

Table 4. The driving factors of intestinal and non-intestinal microbial community assemblies of *Ips* BBs.

fungal unigenes was significantly higher than bacterial genes in both intestinal and non-intestinal samples for the cell growth and death process (Cell cycle - yeast and Meiosis - yeast). However, intestinal and non-intestinal bacterial unigenes related to xenobiotics biodegradation and metabolism process were more abundant than fungal unigenes in both intestinal and non-intestinal samples. The study revealed 15 pathways involved in this process (Supplementary Table S4), encompassing 45, 44 fungal unigenes in the intestinal and non-intestinal samples, and 245, 487 bacterial unigenes in the intestinal and non-intestinal samples (Fig. 4a–d). These unigenes were primarily distributed in *Acinetobacter*, *Erwinia*, *Serratia*, *Mycolicibacterium*, *Mycobacterium* (Supplementary Table S4). Additionally, differential pathways were compared between intestinal and non-intestinal samples of five *Ips* BBs. No significant differences were observed between intestinal and non-intestinal samples for microbes of *I. chinensis*. However, significant variations were existed in multiple pathways for symbiotic microbes in the intestinal and non-intestinal samples of the other four *Ips* BBs. In particular, *I. subelongatus* and *I. hauseri*, distinct discrepancies were found in glutathione metabolism (ko00480), basal transcription factors (ko03022), and purine metabolism (ko00230) pathways. The Glycolysis / Gluconeogenesis) pathway (ko00010) also showed significant differences between intestinal and non-intestinal samples for microbes associated with *I. nitidus* and *I. hauseri* (Fig. 4e, f).

Fungi and bacteria exhibited the highest gene abundances in the intestinal and non-intestinal samples for energy metabolism, particularly oxidative phosphorylation in the non-intestinal samples with higher gene relative abundance compared to that of intestines (Fig. 5a, b). A total of 1,209 unigenes were annotated as being related to this process, including 300 and 395 fungal unigenes in the intestinal and non-intestinal samples. These fungal unigenes were annotated in *Ogataea* and *Wickerhamomyces* (yeasts), *Ophiostoma* and *Ceratocystis* (ophiostomatoid fungi), fungi unclassified, Ascomycota unclassified, and other groups (Supplementary Table S5). The most abundant fungal unigenes were found in non-intestines of *I. subelongatus* (ISC: 58, ICC: 73, ITC: 81, IHC: 85, INC: 89; IS: 150, IC: 74, IT: 110, IH: 83, IN: 85). There were 448 bacterial unigenes in the intestinal samples and 517 bacterial unigenes in the non-intestinal samples. These bacterial unigenes distributed in *Microbacterium* and *Paenarthrobacter* (Micrococcales), *Macrococcus* and *Staphylococcus* (Bacillales), *Erwinia*, Erwiniaceae spp., *Serratia*, *Citrobacter*, *Enterobacter*, and *Escherichia* (Enterobacterales), *Acinetobacter* and *Pseudomonas* (Pseudomonadales) and *Pseudoxanthomonas* (Xanthomonadales). Similarly, the most abundant genes were also found in the non-intestinal samples of *I. subelongatus* (ISC: 115, ICC: 132, ITC: 131, IHC: 93, INC: 133; IS: 247, IC: 85, IT: 144, IH: 81, IN: 93). It was found that gene relative abundance of dominant fungal (Saccharomycetales and Ophiostomatoid fungi) and bacterial (Enterobacterales and Micrococcales) groups involved in oxidative phosphorylation varied with intestinal and non-intestinal in different *Ips* BBs (Fig. 5c, d). These taxa may be crucial microbes for energy metabolism.

Candidate enzymes and unigenes of steroid biosynthesis

Nine enzymes were identified, including four reductases (Delta24-sterol reductase, Delta24 (24(1))-sterol reductase, 7-dehydrocholesterol reductase, and Delta14-sterol reductase), two monooxygenases (calcidiol 1-monooxygenase and methylsterol monooxygenase), one transferase (sterol O-acyltransferase), one lipase (lysosomal acid lipase/cholesteryl ester hydrolase), and one desaturase (sterol 22-desaturase) (Fig. 6a and Supplementary Table S6). There were 55 functional genes involved in the steroid biosynthesis pathway (Ko00100) for this study with 32 fungal unigenes in the intestinal samples and 40 in non-intestinal samples. It exhibited the highest number of fungal unigene in the non-intestinal samples of *I. subelongatus* (ISC: F: 11, B: 2; ICC:

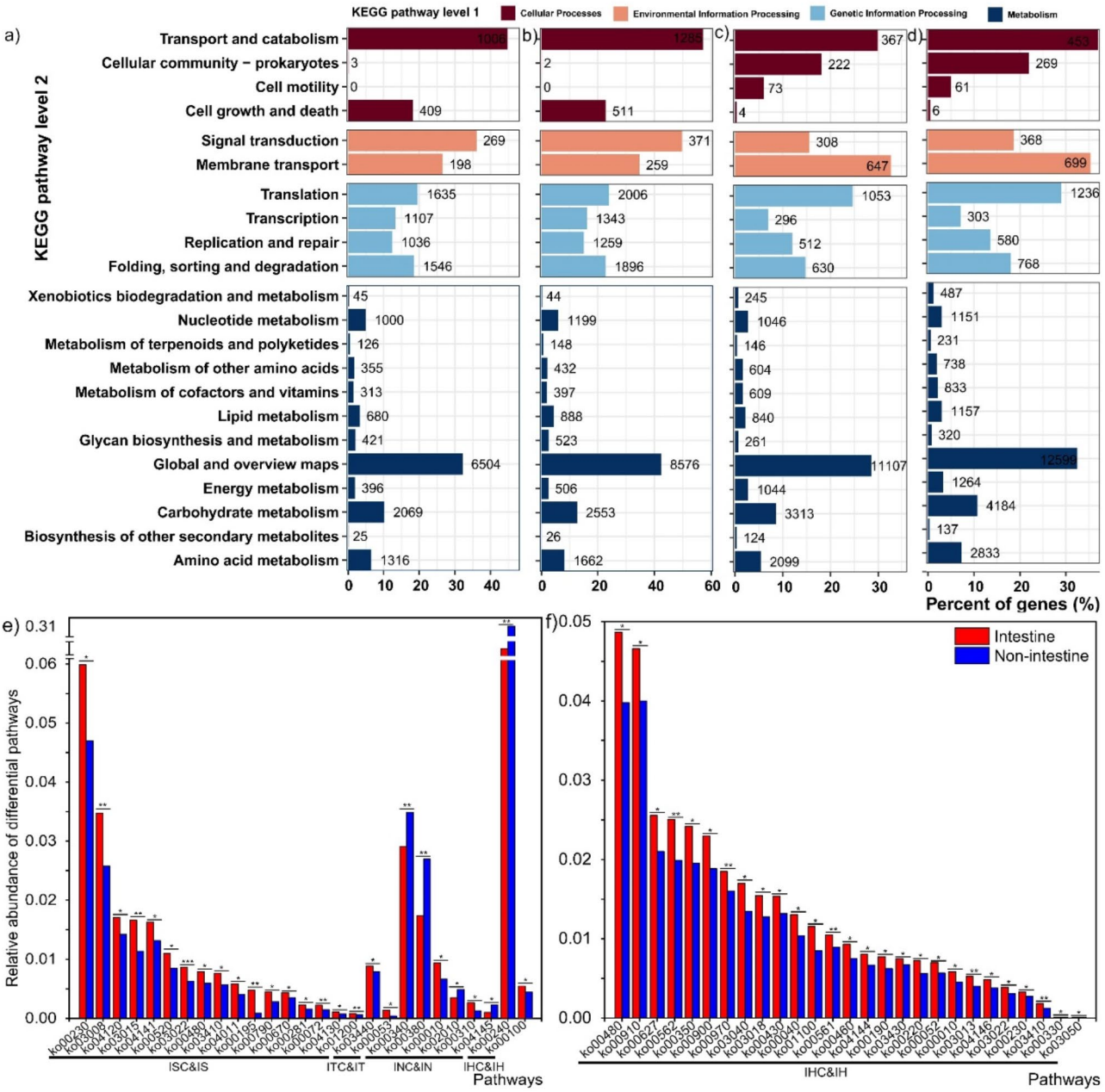

Fig. 4. KEGG enrichment of fungal and bacterial unigenes at KEGG pathway level 2, along with differential pathways in the intestinal and non-intestinal samples of *Ips* BBs. (**a**,**b**) The number of fungal unigenes in the intestinal and non-intestinal samples and (**c**,**d**) bacterial unigenes in the intestinal and non-intestinal samples across different KEGG pathways. (**e**,**f**) Stamp analysis representing the relative abundances of microbial unigenes at differential pathways in the intestinal and non-intestinal samples of *I. subelongatus*, *I. typographus*, *I. nitidus* and *I. hauseri*.

F: 8, B: 2; ITC: F: 14, B: 4; IHC: F: 8, B: 2; INC: F: 4, B: 1; IS: F: 19, B: 3; IC: F: 10, B: 2; IT: F: 15, B: 5; IH: F: 8, B: 2; IN: F: 4, B: 0). These unigenes mainly assigned to Saccharomycetales (unclassified Saccharomycetales, *Wickerhamomyces*, *Candida*, *Ogataea*, *Wickerhamiella* and *Schizosaccharomyces*), unclassified fungi, Mucorales and Methylococcales. Additionally, it was found 9 bacterial unigenes in the intestinal samples and 10 in the non-intestinal samples, which mainly distributed in *Methylomicrobium*, *Enhygromyxa*, *Nannocystis* (Fig. 6a and Supplementary Table S6).

Candidate enzymes and genes of terpene and phenolic compounds degradation

Four enzymes were found to degrade limonene (ko00907) (Fig. 6b). A total of 97 related unigenes were found, including 13 fungal and 49 bacterial unigenes in the intestinal samples, 12 fungal and 64 bacterial unigenes in non-intestinal samples. Notably, the non-intestinal samples of *I. subelongatus* exhibited the most abundant

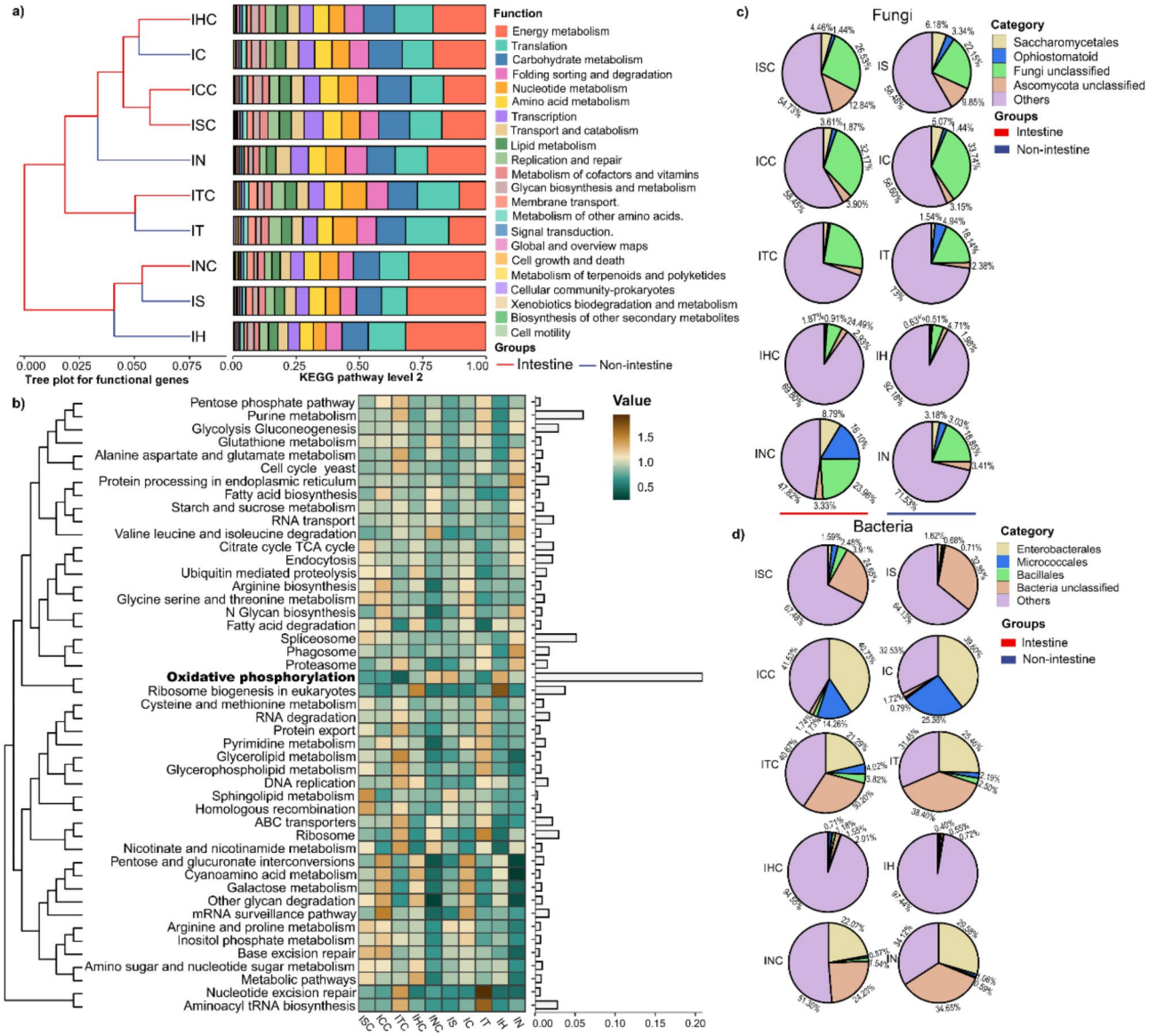

Fig. 5. Gene relative abundances in different intestinal and non-intestinal symbiotic microbial functional pathways of five *Ips* BBs. (**a**) Clustering bar plot of gene relative abundance across KEGG pathways (level 2) in the intestinal and non-intestinal samples of each *Ips* bark beetle. (**b**) Gene relative abundance exceeding 0.003% (genes less than 0.003% are not shown) across KEGG pathway (level 3) in the intestinal and non-intestinal samples of every *Ips* bark beetle. (**c**,**d**) Proportion (gene relative abundance of every dominant taxon occupying the abundance all taxa) of dominant fungi and bacteria involved in oxidative phosphorylation in the intestinal and non-intestinal samples of each *Ips* bark beetle.

unigenes (ISC: F: 5, B: 6; ICC: F: 4, B: 13; ITC: F: 3, B: 9; IHC: F: 5, B: 3; INC: F: 1, B: 20; IS: F:4, B: 31; IC: F: 4, B: 11; IT: F: 3, B: 9; IH: F: 4, B: 3; IN: F: 1, B: 12) (Supplementary Table S6). These unigenes were primarily associated with *Acinetobacter*, *Erwinia*, *Serratia* for this pathway (Supplementary Table S6).

It was found six enzymes and 14 unigenes related to phenolic compounds degradation (Fig. 6c). These related genes belonged to *Acinetobacter* (10 genes), *Serratia* (one gene), *Hafnia* (one gene), and *Mycobacterium* (one gene) in the non-intestinal samples of *I. subelongatus*, and fungal *Kuraishia* (Saccharomycetales) (one gene) in the intestinal samples of *I. nitidus* (Fig. 6c and Supplementary Table S6).

Candidate CAZy enzymes and unigenes of polysaccharides degradation

Fifty-five enzymes belonging to 64 families, were identified to be involve in polysaccharides hydrolysis or degradation (Fig. 6d). A total of 8,107 functional genes were identified with 2,578 fungal unigenes in the intestinal samples and 3,111 fungal unigenes in the non-intestinal samples. Bacterial unigenes totaled 2,943 in the intestinal samples and 3,404 in non-intestinal samples (Supplementary Table S7). These unigenes were predominantly associated with unclassified fungi, unclassified Ascomycota, *Erwinia*, *Acinetobacter*, *Enterobacter*,

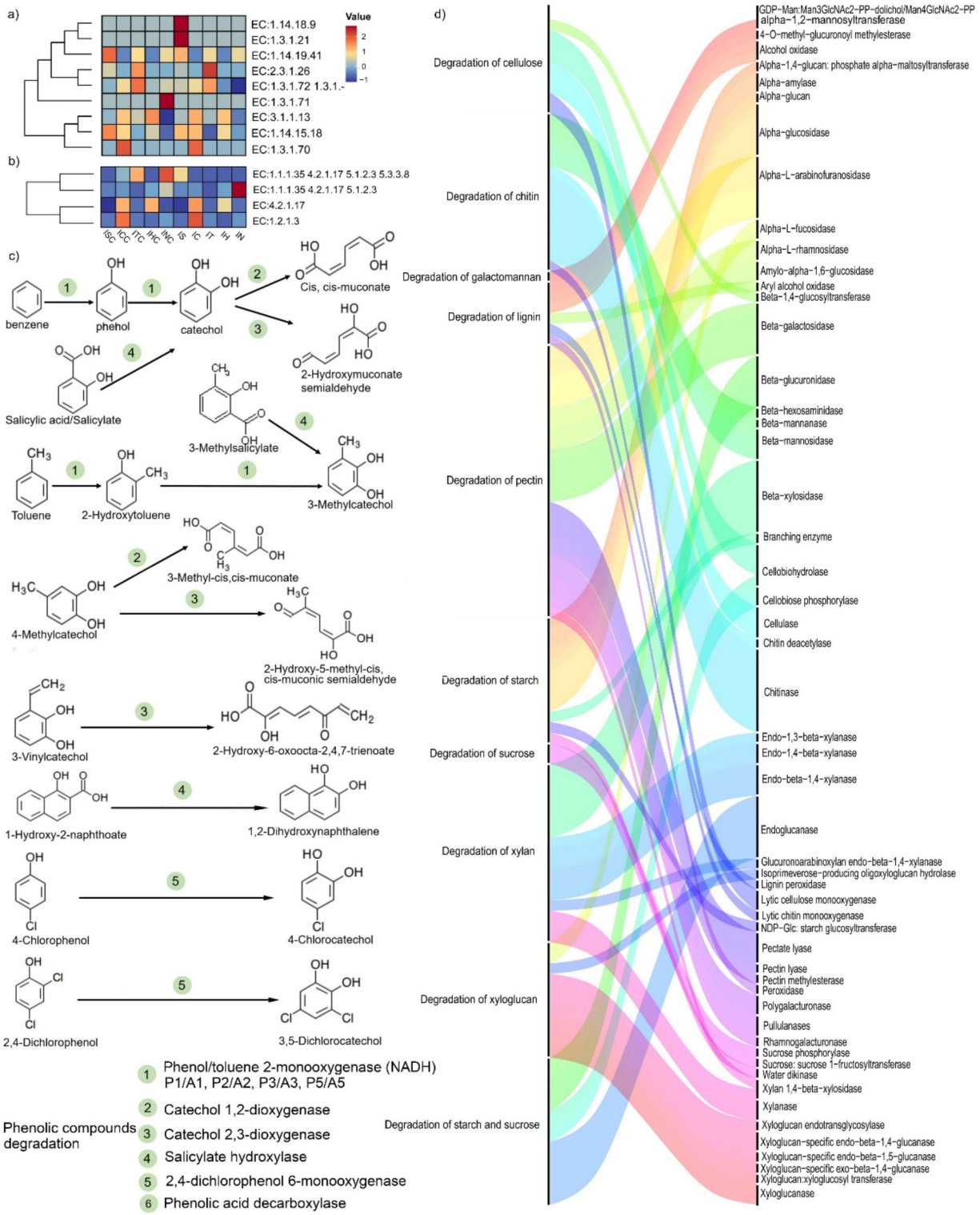

Fig. 6. Candidate functional enzymes and genes related to steroid biosynthesis, aromatic and phenolic compounds degradation, and polysaccharides degradation annotating in the intestinal and non-intestinal samples of *Ips* BBs-associated microbes. Gene abundance and related enzymes of steroid biosynthesis (**a**) and limonene and pinene degradation (**b**) in the intestinal and non-intestinal samples for *Ips* BBs. (**c**) Key chemical reactions and enzymes involved in phenolic compounds degradation. (**d**) Enzymes involved in the degradation of complex compounds (cellulose, chitin, galactomannan, lignin, pectin, xylan, xyloglucan, starch and sucrose) in the intestinal and non-intestinal samples.

Groups	ISC	ICC	ITC	IHC	INC	IS	IC	IT	IH	IN
Fungi	600	720	851	739	520	1155	730	903	682	538
Bacteria	625	849	879	664	738	1521	646	831	596	600

Table 5. The number of fungal and bacterial unigene related to polysaccharides degradation in the intestinal and non-intestinal samples of each *Ips* bark beetle.

Serratia, etc. The most unigenes were harbored in the non-intestines of *I. subelongatus* and intestines of *I. typographus* (Table 5).

Discussion

This study investigated microbial community structures, interactions, and assembly processes in the intestines and non-intestines of five *Ips* BBs. The results revealed the abundance of different microbial taxa varied with hosts, dietary trees and specific habitats, despite a general similarity in community composition between intestinal and non-intestinal samples. Mutualistic interactions among intestinal microbes appeared stronger than those of non-intestinal ones. Functional genes and enzymes involved in the steroid biosynthesis and oxidative phosphorylation were predominantly presented in the non-intestinal fungal samples (Saccharomycetales and ophiostomatoid fungi). In contrast, genes and enzymes related to terpenoids, phenol, and polysaccharides degradation were primarily associated with intestinal bacterial samples (*Acinetobacter*, *Erwinia*, *Enterobacter*, and *Serratia*, etc.). The non-intestinal samples of *I. subelongatus* exhibited the most abundant unigenes for above processes. Understanding the roles of these microbial communities in nutritional provision and complex compound degradation provides deeper insights into the cooperative mechanisms underlying *Ips* BBs growth, development, colonization, and metabolism. This study offers valuable insights into the symbiotic relationships between *Ips* species and their microbial partners.

Comparison of intestinal and non-intestinal microbial community structures

Microbial diversity analysis revealed similar fungal and bacterial community compositions in the intestinal and non-intestinal samples, with variations in the relative abundances of different microbial taxa (Fig. 2a–c). These differences were likely influenced by host species, sampling sites, environmental conditions (season, habitats, climate change), development stage and diets[7,8,10,15,51,66]. Ascomycota was most abundant fungal community in both habitats, aligning with previous studies on *Ips* species and *Polygraphus poligraphus* Linnaeus[9], galleries of *Megaplatypus mutatus* Bright & Skidmore[67] as well as other BBs[26]. Fungal taxa such as Erysiphales, Glomerales, Mucorales, Saccharomycetales and ophiostomatoid fungi[9,15] were found frequently in this study, though with some regional variations compared to studies on *Ips* BBs in the Czech Republic[9], *Dendroctonus armandi* Tsai & Li galleries[68] in China, and Platypodinae species in Argentina[67]. Yeasts were particularly abundant in the intestinal and non-intestinal samples of spruce-feeding BBs compared to other dietary BBs. This was inconsistent with findings by Chakraborty[3], who noted that this taxon had higher abundances in pine-feeding beetle (*Ips acuminatus* Wood & Bright). For α-diversity, it showed that fungal-symbionts were more abundant in the intestinal samples than in the non-intestinal ones. The bacterial communities of BBs were dominated by Pseudomonadota, Actinobacteria, Firmicutes, and Bacteroidetes, consistent with the results of previous studies on the components of symbiotic bacteria for beetles[7,9,69,70]. Within these communities, Enterobacterales, Pseudomonadales, and Bacillales exhibited higher diversity and relative abundances in the intestinal samples for this study. The results have been proved in the intestines of *I. typographus*[15] and *Samia ricini* Anderson[20]. *Enterobacter* showed higher abundance in the intestinal and non-intestinal samples of *I. chinensis* (pine-feeding bark beetle) than that of other dietary beetles. *Serratia*, *Erwinia* had higher relative abundance in the intestinal than in the non-intestinal samples of *I. chinensis* (pine-feeding bark beetle), *I. hauseri* and *I. nitidus* (spruce-feeding BBs). It has been confirmed that *Serratia*, *Erwinia* and *Enterobacter* were more abundant in intestinal samples of *Ips sexdentatus*, *Ips acuminatus*, *Ips duplicatus* and *Curculio chinensis*[8,71]. *Pseudomonas* and *Staphylococcus* showed higher relative abundances in the intestinal samples of different dietary *Ips* BBs compared to those in the non-intestinal samples. These microbes were common taxa in the intestinal samples of BBs[8,9]. Additionally, interaction among the microbes affected microbial community structures and functionality[36,40]. In our study, mutualistic interrelationship in the intestinal microbes may be more complex than in the non-intestinal samples. *Pantoea* and *Serratia* only presented in the intestinal network interaction, indicating that these two genera may exhibit significant effects on the intestinal function. *Pantoea*, previously isolated from wood-boring beetle intestines, was known to fix nitrogen[10,70].

Comparison of intestinal and non-intestinal microbial assemblies

Despite ongoing debates about the driving mechanisms of deterministic versus stochastic processes, our findings revealed distinct disparities between intestinal and non-intestinal environments. Intestinal tracts, enriched with abundant bacterial symbionts, are crucial for host digestion, detoxification, and transmission across generations for host adaptation[5,6]. Non-intestinal samples primarily carry or transmit symbionts for nutrition provision to host beetles[3]. In our work, HD and UP of the stochastic process may be the main driving force in the intestinal core microbial community assemblies. However, in the non-intestinal microbial assembly was governed by HS of the deterministic process, which may play more a critical role in maintaining the similarity and stability of the non-intestinal microbial communities. This finding aligned with previous research on *Dicranocephalus wallichii bowringi* Pascoe, honeybee *Apis cerana* Fabricius and *A. mellifera*, which highlighted that DL and drift

influenced intestinal microbial community assemblies. However, HOS contributed to *Apis mellifera* intestinal microbial assembly due to unconsidered host species and geographical sites. Microbial community assemblies varied with surroundings, dietary trees, and host species[42,49].

Nutritional provision deriving from non-intestinal fungi symbionts

Non-intestinal fungi have played pivotal roles in the survival and development of BBs[3,24,72–74]. In this study, gene abundance of oxidative phosphorylation process was the highest, particularly in the non-intestines of *I. subelongatus*. These fungal unigenes were found in *Ogataea* and *Wickerhamomyces* (Saccharomycetales) and *Ophiostoma* and *Ceratocystis* (ophiostomatoid fungi). Previous investigation of *I. nitidus* chromosome-level assembled genome confirmed that unigenes were enriched in energy metabolism[32]. It may be speculated that microbes and insect hosts were conjointly likely to involve in energy metabolism to overcome inhospitable environment, benefiting insects alone or both. However, detailed symbiotic mechanisms between *Ips* BBs and symbionts remained poorly understood. Fungal symbionts are keystone elements for steroid biosynthesis in BBs, with 40 fungal unigenes and seven enzymes in non-intestinal samples in this study. These genes were mainly classified into *Candida*, *Ogataea*, and *Wickerhamiella*. Previous studies have found that yeasts and other fungi serving as nitrogen sources have significant effects on hosts colonization and reproduction[24,26,44,73–75]. Additionally, the number of fungal unigenes was clearly higher than bacterial unigenes in the cell growth and death pathways (Cell cycle-yeast and Meiosis-yeast), suggesting that yeast symbionts have profoundly influence on biochemical signals sense, DNA damage and repair. *I. nitidus* genome analysis revealed that gene expression involved in DNA replication and repair was also greater, implying a cooperative role may exist between BBs and yeast symbionts. Ophiostomatoid fungi contain abundant ergosterol (sterol), which is essential for the development of eggs, larvae and pupae in BBs[73,76]. *Ophiostoma* and *Ceratocystis* exhibited higher gene abundance in the non-intestinal samples. Ophiostomataceae is regarded as an essential nitrogen source for the growth and development of Scolytinae beetles[73]. The fungi from this family Ceratocystidaceae, are able to detoxify defensive compounds warding off attacks from beetles or fungi to adapt and survive on a wide array of host trees, especially, this genus *Ceratocytosis*[77,78]. These symbionts in both two families and their associated insects can build novel destructive interrelations, posing hazards to health of forest agriculture in the long-term development[79]. Generally, yeasts and ophiostomatoid fungi may be closely related to nutrient metabolism for BBs. Nutrition provided by non-intestinal fungal symbionts may complement the metabolic capacities, fostering co-evolution within the symbiotic system.

Degradation of complex compounds by intestinal bacteria associates

Terpenoids and phenolics serve as defensive compounds by resisting bark beetles' attacks and inhibit host colonization[21,69,80,81]. In this study, four enzymes were found to be involved in terpene degradation, which belonged to intestinal bacteria associated with five *Ips* BBs. These enzymes are capable of transforming α-pinene into 3-isopropylbut-3-enoic acid or converting geraniol to 5-Methyl-3-oxo-4-hexenoyl-CoA and take (-)-(s) limonene into perillic acid. The results are similar to previous enzymatic annotations of microbes associated with *Dendroctonus ponderosae* Hopkins, but there are slight variations in the distribution of genes related to this pathway, which belong to *Pseudomonas*, *Rahnella*, *Serratia*, and *Stenotrophomonas*[69]. Catechol, which consists of a benzene ring with adjacent hydroxyl groups, is regarded as fundamental structural unit of phenolic compounds. Enzymes (e.g. catechol dioxygenases) associated with it can effectively catalyze phenolics as carbon sources to enhance adaptability for host colonization[82]. These enzymes (Catechol 1,2- dioxygenase and Catechol 2,3- dioxygenase) were also found in our study.

Carbohydrases, including GH1, GH5, GH9, GH28, GH32, GH45, GH48, CE8, and PL4, play crucial roles in degrading complex compounds[32,83]. GHs have been annotated in *Grosmannia clavigera* (Rob.-Jeffr. & R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf. associated with *D. ponderosae* and microbes associated with *Anoplophora glabripennis* Motschulsky, which degrade polysaccharides[19,84]. In this study, 26 GHs, including 18 enzymes and 1,563 unigenes in the intestinal bacterial symbionts associated with *Ips* BBs, were found to be involved in degrading cellulose, xylan, xyloglucan, pectin and chitin (Supplementary Table S7). GH1 possessed 231 genes, which was the most abundant in polysaccharide metabolism. It was also found in the intestine of *A. glabripennis*, assisting to hydrolyze cellobiose[19]. We found GH9 may degrade cellulose, xyloglucan, starch and sucrose in this study. However, it was almost rare in BBs, such as *I. nitidus*, *I. typographus*, *D. ponderosae* and *Hypothenemus hampei* Ferrari[32]. Additionally, GH32 could convert sucrose into glucose and fructose[85], which was only in *D. ponderosae*[32]. In our study, CE8, GT4, GT5 and GT26 may involve in degrading pectin, galactomannan, starch, and cellulose. Previous studies detected that these enzymes played an important role in regulating functionality of insects[86]. In addition, GH4, GH31, GH13, GH63, GH76, PL1, PL3 and PL9 were also found in bacterial symbionts associated with *Ips* BBs. It was speculated that microbes may help BBs degrade complex compounds.

These unigenes related to complex compounds were principally categorized into intestinal unclassified bacteria, *Erwinia*, *Acinetobacter*, *Enterobacter*, and *Serratia* (Supplementary Table S7), which are essential microbial taxa for BBs. Previous studies have been confirmed that most *Acinetobacter*, *Pseudomonas*, and *Rahnella* can promote host insect to degrade complex compounds[20,63,71]. In our study, bacterial unigenes in the intestinal samples were found to be far superior to non-intestinal fungal unigenes for every pathway of xenobiotics biodegradation and metabolism, indicating intestinal bacteria may make significant contributions to degrading complex substances. For example, unigenes involved in xenobiotics biodegradation and metabolism were greatly abundant in microbes associated with *I. acuminatus*[8]. Particularly, the benzoate degradation pathway, which possessed the highest number of unigenes in the xenobiotic detoxification, was crucial for detoxifying a variety of toxic compounds including phenols and benzaldehyde, which are common secondary metabolites in plants

that hinder the attacks of bark-feeding pests. In sum, intestinal bacteria collaborated with BBs overcome toxic complex compounds from coniferous trees, facilitating colonization successfully.

Conclusions

The microbial community assembly mechanisms and functional structures were studied in the intestinal and non-intestinal samples of five *Ips* BBs using a metagenomic approach. Our findings demonstrated that hosts beetles, dietary trees, and specific habitats (intestines vs. non-intestines) significantly influenced microbial community compositions. Network analysis showed a tighter mutualistic symbiosis in the intestinal microbial communities compared to the non-intestinal ones. The core microbial community assemblies in the intestines were primarily driven by HD and UP, whereas HS was primary driver in the non-intestinal microbial community assemblies. This analysis provides fundamental insights into the spatial patterns of microbial communities in BBs. Additionally, Saccharomycetales and Ophiostomatoid fungi may serve as dominant core non-intestinal fungal communities to cope with nutritional deficiencies. *Erwinia*, *Acinetobacter*, *Enterobacter*, *Serratia*, and others may be served as crucial intestinal bacterial taxa by degrading or detoxifying defensive compounds, helping BBs survive in hostile environments.

Data availability

The raw data of metagenomic sequencing with SRAs (*Ips subelongatus* (SRR30159481-SRR30159486), *Ips chinensis* (SRR30172494-SRR30172499), *Ips typographus* (SRR30172520-SRR30172525), *Ips hauseri* (SRR30186660-SRR30186665), and *Ips niditus* (SRR24210634-SRR24210639) are available under NCBI.

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Declarations

Competing interests

The authors declare no competing interests.

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