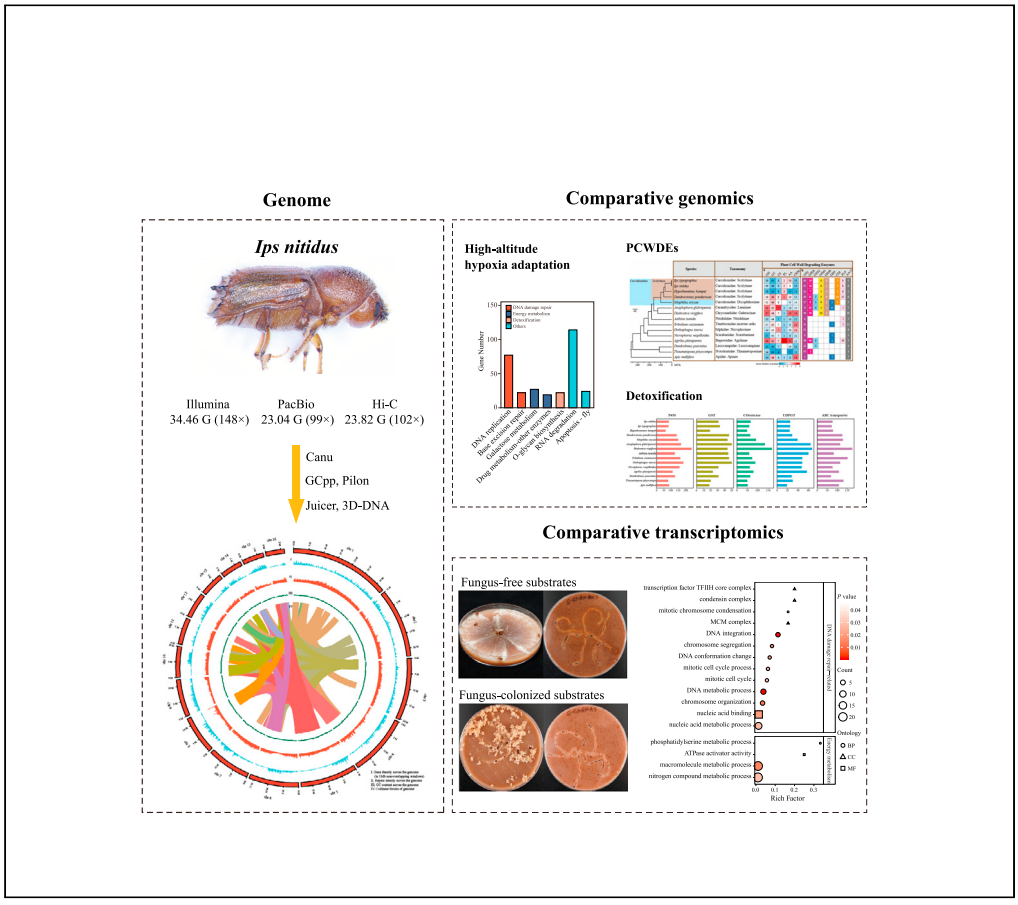
iScience

CellPress
OPEN ACCESS

Article

Genome and transcriptome of *Ips nitidus* provide insights into high-altitude hypoxia adaptation and symbiosis



Zheng Wang, Ya Liu, Huimin Wang, ..., Fuzhong Han, Xingyao Zhang, Quan Lu

hxliu722@126.com (H.L.)
luquan@caf.ac.cn (Q.L.)

Highlights

A chromosome-level genome of spruce pest inhabiting the Tibetan Plateau, *I. nitidus*

Comparative genomics reveals potential high-altitude hypoxia adaptation in *I. nitidus*

PCWDEs and detoxification-related gene families were analyzed

Fungal symbiont may assist *I. nitidus* in better coping with hypoxia and detoxifying

Wang et al., iScience 26, 107793
October 20, 2023 © 2023
https://doi.org/10.1016/j.isci.2023.107793

iScience

CellPress
OPEN ACCESS

Article

Genome and transcriptome of *Ips nitidus* provide insights into high-altitude hypoxia adaptation and symbiosis

Zheng Wang,[1,2] Ya Liu,[1] Huimin Wang,[1] Amit Roy,[3] Huixiang Liu,[2,*] Fuzhong Han,[4] Xingyao Zhang,[1] and Quan Lu[1,5,*]

SUMMARY

***Ips nitidus* is a well-known conifer pest that has contributed significantly to spruce forest disturbance in the Qinghai-Tibet Plateau and seriously threatens the ecological balance of these areas. We report a chromosome-level genome of *I. nitidus* determined by PacBio and Hi-C technology. Phylogenetic inference showed that it diverged from the common ancestor of *I. typographus* ~2.27 mya. Gene family expansion in *I. nitidus* was characterized by DNA damage repair and energy metabolism, which may facilitate adaptation to high-altitude hypoxia. Interestingly, differential gene expression analysis revealed upregulated genes associated with high-altitude hypoxia adaptation and downregulated genes associated with detoxification after feeding and tunneling in fungal symbiont *Ophiostoma bicolor*-colonized substrates. Our findings provide evidence of the potential adaptability of *I. nitidus* to conifer host, high-altitude hypoxia and insight into how fungal symbiont assist in this process. This study enhances our understanding of insect adaptation, symbiosis, and pest management.**

INTRODUCTION

The Qinghai-Tibet Plateau is the largest and highest plateau in the world.[1,2] High-altitude extremes in the Qinghai-Tibet Plateau are characterized by hypoxia, intense ultraviolet radiation, and severe coldness, which provide a "natural laboratory" to study adaptive evolution. Genome-level studies of animals have been explored from this region in the last decade, mainly focused on humans,[3] mammals,[4–8] birds,[9] reptiles,[10] amphibians,[11] and fish.[12] High-altitude animals commonly exhibit positive selection and rapid evolution of genes involved in hypoxia responses, DNA damage repair, and energy metabolism, suggesting that general genetic mechanisms might be utilized to adapt to high-altitude extremes. However, except for the study on Tibetan migratory locusts in the Qinghai-Tibet Plateau,[13] there are no genome-based studies on the adaptive evolution of insects in the Qinghai-Tibet Plateau, which provides a challenging habitat for insects, the most speciose group of animals.[14]

In the context of climate change, forest pest outbreaks may become more frequent and intense due to more favorable breeding temperatures that cause widespread tree mortality and an increase in carbon emissions.[15–19] Large-scale outbreaks of bark beetles are considered to be one of the major factors contributing to forest disturbance in North America and Europe, causing economic losses comparable to those caused by natural disasters such as storms and fires.[20–24] Severe depletion of conifers due to bark beetle outbreaks or range expansion may cause trajectories outside the resilience limits of forest ecosystems, resulting in irreversible ecosystem regime shifts. So far, *Ips* (Coleoptera: Curculionidae: Scolytinae) is one of the most extensively studied bark beetle genera because of the severe damage they cause to coniferous forests and plantations. There are 37 species of *Ips* worldwide, all distributed in the coniferous forests of the Northern Hemisphere, living in the phloem and cambium for most of their life and utilizing mainly *Pinus* and *Picea* species as a food resource for larvae and adults.[25] They perform host selection mainly by sensing plant volatiles.[26,27] The mass attack mediated by the aggregation pheromone produced by male beetles is one of the crucial factors for their success in breaking through conifer defenses.[28–30] The most destructive species is *Ips typographus*, which is responsible for the decimation of vast spruce forests in Europe,[31] China,[32,33] and Japan.[34] Whole-genome sequencing of this beetle detected expanded gene families involved in plant cell wall degradation and provided insights into forest pest control under global warming.[35] *Ips nitidus*, a sister species of *I. typographus*, is distributed in a small area encompassing the Qinghai-Tibet Plateau of China[25,36]

[1]Key Laboratory of Forest Protection of National Forestry and Grassland Administration, Ecology and Nature Conservation Institute, Chinese Academy of Forestry, Beijing 100091, China
[2]Shandong Research Center for Forestry Harmful Biological Control Engineering and Technology, College of Plant Protection, Shandong Agricultural University, Tai'an 271018, China
[3]Faculty of Forestry and Wood Sciences, EXTEMIT-K and EVA.4.0 Unit, Czech University of Life Sciences, Kamýcká 1176, Prague 6, 165 00 Suchdol, Czech Republic
[4]Maxiu Forest Farm, Huangnan 811300, China
[5]Lead contact
*Correspondence: hxliu722@126.com (H.L.), luquan@caf.ac.cn (Q.L.)
https://doi.org/10.1016/j.isci.2023.107793

Figure 1. *Ips nitidus* and its damage to the spruce forest

(A and B) Adult *I. nitidus* side and front view.

(C) Spines of *I. nitidus*.

(D) The pupa is surrounded by sticky conidia masses produced by fungal symbionts in the pupal chamber.

(E) A close-up of the fungal symbionts.

(F) Galleries of *I. nitidus* in the phloem of *Picea crassifolia*.

(G) Damage at landscape scale of *P. crassifolia* forest. The bare land was once covered with thick spruce forests, but only a faint pile remains after being harvested by *I. nitidus*.

(H) Forestry workers tried to restore spruce forests in damaged areas by establishing nursery plants and replanting, but spruce trees in nursery plants are sometimes killed.

(I) Due to the complex geographical environment of the primeval forest, blocked roads, and the nature reserve policy, local forestry workers can only reduce the density of the next generation of *I. nitidus* by artificial bark peeling, with limited control effect. Note: (A–C) are modified from Wang et al.[36]

and has also caused substantial destruction of the spruce forest.[37] Except during the search for a new host as an adult, *I. nitidus* spends its entire life cycle inside tree cambium and completes one (above 3,000 m elevation) or two generations (within 2,800–3,000 m elevation) per year.[37–39] Due to the unique high-altitude environment in and around the Qinghai-Tibet Plateau, the growth of primary tree species is very slow, and most often, the damage due to bark beetle attacks is irreversible here (Figures 1G–1I). Therefore, *I. nitidus* poses a significant threat to the fragile spruce habitats of the Qinghai-Tibet Plateau. Since the 1970s, climate warming and increasing drought may have contributed to

frequent bark beetle outbreaks.[40,41] Although forest managers have tried various direct and indirect methods to manage *I. nitidus*,[42–44] infestations will continue due to the availability of susceptible forests and favorable climatic conditions.[45] Thus, there is an urgent prerequisite for developing superior methods for aggressive *I. nitidus* management.

The molecular arms race between insects and plants has been ongoing since the Ordovician period, approximately 479 million years ago.[14,46] The diversity of herbivorous insects is proposed to be correlated to the diversity of the plants on which they thrive.[47,48] Long periods of coevolution between herbivorous insects and plants have led to the development of a formidable defense system in plants, while herbivorous insects have evolved traits to overcome those defensive responses.[49,50] It is estimated that extant conifers and bark beetle species coexisted for at least three million years,[51] providing ample time for mutual selection and adaptation.

Bark beetles are exposed to defensive compounds produced by conifers at all life stages since egg-laying occurs within the phloem and cambium of the host tree. When attacked by bark beetles, conifers produce large amounts of oleoresin-containing terpenoids, phenolics, and other compounds that are entomotoxic to reduce beetle populations.[52–56] Over time, bark beetles have evolved to counteract the oleoresin-derived chemical defenses of the conifer by synthesizing aggregation pheromones.[54,56] A pheromone-mediated mass attack overwhelms the defenses of the conifer host due to the high number of beetle invaders.[57] Some genes related to olfactory detection and pheromone synthesis in bark beetles have been elucidated recently.[57–60] Genomic studies of the mechanisms by which bark beetles overcome coniferous host defenses are still limited. Thus, it is optimal to extend our effort to enrich the available genomic resources of conifer-killing bark beetles and delineate the molecular fine-tuning of high-altitude host usages within economically important *Ips* bark beetles. This initiative can facilitate functional genomics research and lead to novel formulations for their management. Species-specific RNA interference (RNAi)-based forest protection products (FPPs), for example, can be developed for bark beetle management in future using sequence information from the genome.[61]

Many insects, including bark beetles, have upheld a steady symbiotic relationship with microorganisms for generations, and they act as a holobiont against environmental challenges.[41,62–66] Ambrosia beetles have even developed specialized pockets called mycangia to preserve such precious symbiotic associations.[67,68] Fungal symbionts are thought to play a vital role in the success of conifer bark beetle colonization.[69–71] Bark beetles uphold symbiotic relationships with a diverse group of fungi, with the most well-known species belonging to the ophiostomatoid fungi, which belong to the orders Ophiostomatales (Sordariomycetidae, Sordariomycetes, Ascomycota) and Microascales (Hypocreomycetidae, Sordariomycetes, Ascomycota).[72–74] The classic paradigm that phytopathogenic fungi associated with tree-killing bark beetles are critical for overwhelming tree defenses and killing host trees has driven research on the symbiosis of fungi and bark beetles for decades. However, Six and Wingfield[75] challenged this idea and suggested that apart from helping bark beetles to kill the host tree, fungi perform other important roles, such as nutrient provisioning,[76–79] detoxification of tree defenses,[80,81] synthesis of bark beetles' semiochemicals,[82–85] and exclusion of pathogens or other antagonistic microbes of insects.[77,86,87] In any case, the previously described studies are confined to phenomenological observations or metabolomics and lack genomic evidence.

The present study reports a chromosome-level assembly and annotation of *I. nitidus* genome (Figure 2). The expansion and contraction of gene families related to high-altitude hypoxia adaptation, plant cell wall-degrading enzymes (PCWDEs), and detoxification were analyzed by comparing the genome with other related species. Moreover, the orchestration of gene regulation for detoxification and hypoxic response during feeding and tunneling in semi-natural substrates delivered insights into beetle symbiosis with fungi. This study provides a solid foundation for understanding insect adaptation to extreme environments and a new perspective for determining the symbiotic association between bark beetles and their fungal associates. Such an in-depth understanding of *I. nitidus* physiology and symbiosis will further facilitate the formulation of ecologically relevant testable hypotheses and downstream functional studies, aiding robust understanding and management strategies.

RESULTS

Genome sequencing and assembly

In total, we generated 34.46 Gb of clean data by Illumina sequencing, which represented an estimated 148-fold coverage of the *I. nitidus* genome (Table 1). The genome size was approximately 205.44 Mb with a heterozygosity rate of 1.25% based on k-mer analyses (Figure S1; Table S1). A quality check did not reveal any microbial contamination (Figure S2), and for long-read sequencing, we obtained 23.04 Gb PacBio reads after filtering low-quality sequences, representing a 99-fold coverage of the *I. nitidus* genome (Table 1). An initial *de novo* assembly of 375.70 Mb with a contig N50 of 99.16 kb was generated (Table 2). The average GC content was 35.14%. After potentially redundant contigs were discarded based on sequence similarities, a 280.38 Mb assembly with contig N50 of 283.31 kb was generated for *I. nitidus* (Table 2). A total of 93.42% of Illumina reads and 84.68%–91.28% of transcriptome sequencing reads were mapped to the genome, respectively (Tables S2 and S3). We obtained 23.82 Gb of clean data (158,810,286 paired-end reads) filtered from 24.72 Gb of Hi-C raw data (164,802,671 paired-end reads) (Table 1). The 231.42 Mb genome was assembled from 212 scaffolds with an N50 of 16.44 Mb (Table 2). A total of 225 Mb assembled sequences were anchored onto 16 pseudochromosomes representing 97.2% genome sequence coverage (Figures 2B and 2C). This chromosome number corresponds to the sister species, *I. typographus*,[88] and another species within *Ips* genus, *I. pini*.[89] Furthermore, the completeness of the genome was assessed using BUSCO, resulting in 1329 (97.2%) complete, 8 (0.6%) fragmented, and 30 (2.2%) missing genes in the insecta_odb10 (Table S4). These results support the accuracy and completeness of the high level of *I. nitidus* genome assembly.

Gene annotation

The genome was annotated with 46.08% repeat sequences, including 2.76% DNA transposons, 4.43% LINEs, 6.00% LTR elements, and 32.35% unclassified elements (Table S5). A total of 27,202 protein-coding genes were annotated in the *I. nitidus* genome by combining three analysis

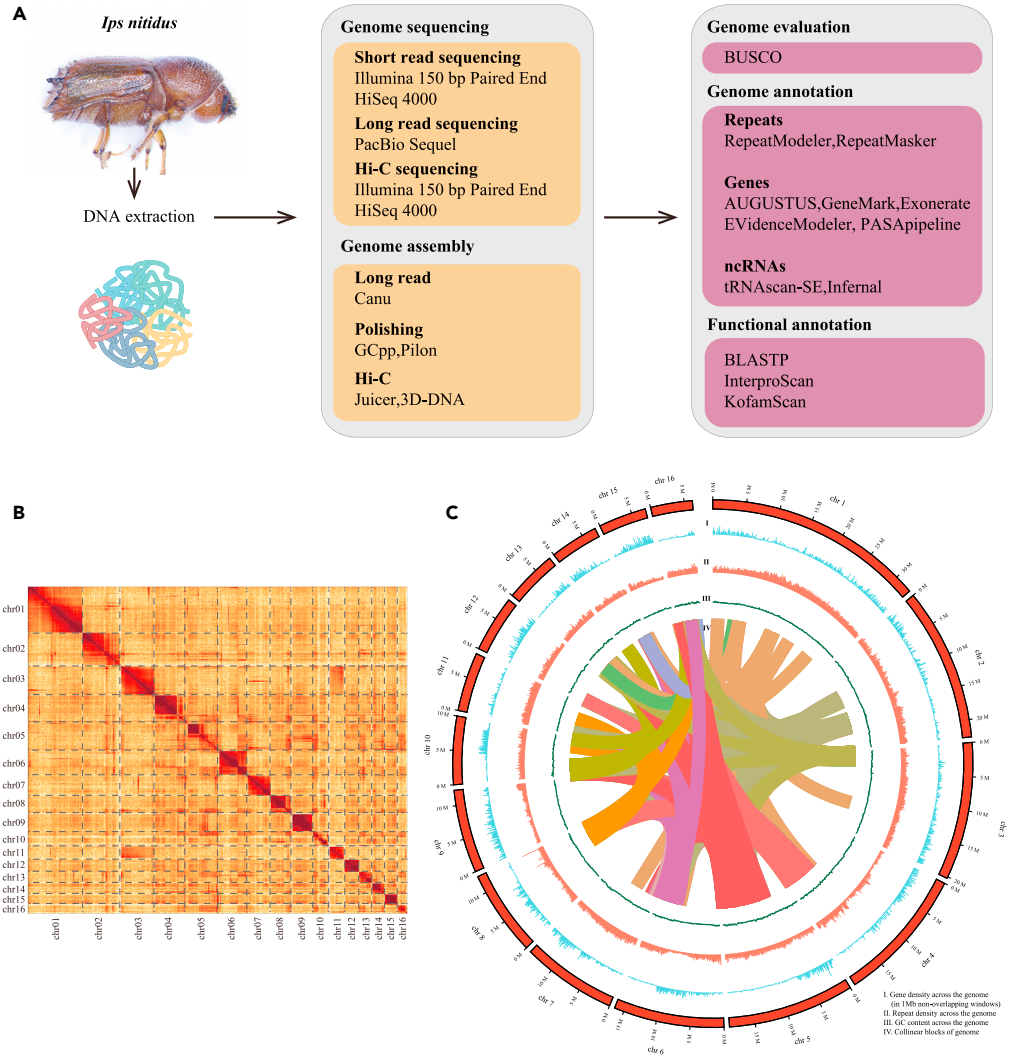


Figure 2. Chromosome-level assembly of *Ips nitidus*

(A) A flowchart for genome assembly and gene annotation of *Ips nitidus*.

(B) Hi-C contact map of the *I. nitidus* genome.

(C) Circos plot showing the genomic landscape of the 16 *I. nitidus* chromosomes. The circles from the outside to the inside represent: I, Gene density across the genome in 1 Mb non-overlapping windows; II, Repeat density across the genome in 1 Mb non-overlapping windows; III, GC content across the genome in 1 Mb non-overlapping windows; IV, Collinear blocks of the genome.

methods: *de novo* (GeneMark: 22,427 genes; Augustus: 32,257 genes), homology (Exonerate: 23,743 genes), and transcriptome based (Table 3). BUSCO analyses assessed the completeness at the gene level as 94.7% (single-copy genes: 88.1%, duplicated genes: 6.6%) in insecta_odb10 (Table S4). The average lengths of genes, CDs, exons, and introns were 4,103.85, 1357.80, 290.87, and 806.13 bp, respectively. The average number of exons per gene was 4.67. A total of 25,986 genes were functionally annotated from nine databases, accounting for 95.53%

Table 1. Sequencing statistics generated for *Ips nitidus* genome assembly

Platform	Library size	rawReads	cleanReads	Raw data (Gb)	Clean data (Gb)	Coverage (X)
Illumina	350 bp	241,186,011	229,700,963	36.18	34.46	148
PacBio	40 kb	1,407,583	325,620	–	23.04	99
Hi-C	600 bp	164,802,671	158,810,286	24.72	23.82	102

Note: A dash indicates data not available.

Table 2. Genome assembly statistics of *Ips nitidus*

Features	PacBio Initial	PacBio Final	PacBio + Hi-C
Total assembly size of contigs (bp)	375,702,926	280,375,318	–
Number of contigs	4,784	2,126	–
N50 contigs length (bp)	99,157	283,305	–
N90 contigs length (bp)	30,764	42,143	–
Longest contigs (bp)	3,137,723	3,169,417	–
Total assembly size of scaffolds (bp)	–	–	231,417,818
Number of scaffolds	–	–	212
N50 scaffolds length (bp)	–	–	16,438,241
N90 scaffolds length (bp)	–	–	7,158,500
BUSCO (%)	–	96.5	97.2

Note: "-" indicates data not available.

of the total number of predicted protein-coding genes in *I. nitidus* (Table 4). In addition, four types of noncoding RNAs were identified and annotated, including 298 rRNAs, 45 snRNAs, 60 miRNAs, and 233 tRNAs (Table 5).

Rapidly expanded and positively selected genes promoting *I. nitidus* high-altitude hypoxia adaptation

The topological structure of the phylogenetic analysis of *I. nitidus* and 14 other species was consistent with previous results (Figure 3A).[14,48] The two *Ips* species diverged approximately 2.27 mya after the range measured with the uplift time of the Qinghai-Tibet Plateau.[90] A total of 611 expanded and 198 contracted gene families were detected in *I. nitidus* after divergence from the ancestor of *I. typographus*. Gene Ontologyand KEGG enrichment analyses of expanded gene families revealed abundant terms or pathways that may be associated with hypoxia adaptation, such as DNA damage repair and energy metabolism (Figures 3B and 3C; Tables S6 and S7). Based on differentially expressed gene (DEG) analysis of the transcriptome data from adult *I. nitidus* exposed to high-altitude hypoxia and low-altitude normoxia (GGQA vs. DGQA), 119 upregulated genes and 155 downregulated genes were detected, among which upregulated genes were also mainly enriched in the previously described two functionally related categories (Figure S3; Table S8). We then analyzed the expression of genes from seven KEGG pathways in different treatments and found that genes from the "DNA replication" (Figure 3D) and "Galactose metabolism" pathways were generally upregulated at high altitudes (GGQA) (Figure 3E), while genes from the other five pathways showed no difference or irregularity. Moreover, we conducted a positive selection analysis on the single-copy orthologs among four Curculionidae species (*I. nitidus*, *I. typographus*, *Dendroctonus ponderosae*, and *Sitophilus oryzae*). A total of 30 genes were identified as positively selected (Table S9). GO enrichment analyses revealed that genes are enriched for several categories related to biosynthetic and metabolic processes (Table S10).

PCWDEs

We annotated the PCWDEs encoded in 15 species genomes, among which that of *Anoplophora glabripennis* were the most abundant (121 families), and the other beetles were between 111 and 119 (Figure 4; Table S11). For *I. nitidus*, a total of 117 PCWDE families containing 649 proteins were annotated, including 40 glycoside hydrolase (GH) families contained 193 proteins, 46 glycosyltransferase (GT) families contained 218 proteins, 8 carbohydrate esterase (CE) families contained 58 proteins, 2 polysaccharide lyase (PL) families contained 8 proteins, 8 auxiliary activity (AA) families contained 96 proteins, and 13 carbohydrate-binding module families contained 76 proteins (Tables S11 and S12). It is worth noting that although a similar number of GH and GT families have been annotated in the genomes of *I. nitidus* and *I. typographus* (40 GH families vs. 38 GH families; 46 GH families vs. 45 GH families), *I. nitidus* has much more GH and GT proteins than *I. typographus* (193 GH proteins vs. 178 GH proteins; 218 GT proteins vs. 200 GT proteins).

Table 3. Statistics for predicted protein-coding genes of *Ips nitidus*

Gene set	Software	Total number of genes	Average gene length (bp)	Average CDS length (bp)	Average exons number per gene	Average exon length (bp)	Average intron length (bp)
De novo	GeneMark	22,427	4,237.40	1,268.76	5.22	242.89	702.84
	Augustus	32,257	3,884.20	1,352.60	4.40	307.32	744.06
Homology	Exonerate	23,743	5,579.32	1,418.37	5.13	289.23	949.34
Final set	PASApipeline	27,202	4,103.85	1,357.80	4.67	290.87	806.13

Table 4. Functionally annotated protein-coding genes

Type		Number	Proportion (%)
Annotation	SwissProt	15,032	55.26
	UniRef50	23,633	86.88
	KEGG	21,217	78.00
	KOG	21,476	78.95
	GO	12,394	45.56
	InterProScan	23,506	86.41
	NR	23,634	86.88
	CAZy	639	2.35
	PFAM	16,623	61.11
Total	Annotated	25,986	95.53
	Gene	27,202	–

Detoxification

Frequent turnover of protein domain numbers is considered the primary mechanism of adaptive divergence in closely related species.[7,9,91,92] In addition to the expansion of the gene family associated with the detoxification metabolic pathway in *I. nitidus* genome (Figure 3C), we also annotated five major detoxification gene families in 15 insect species using the Pfam database, including the cytochrome P450 gene family (P450), glutathione S-transferase (GST), carboxylesterase (COE), UDP-glucuronosyl-transferase (UDPGT), and ATP-binding cassette transporter (ABC transporter) (Figure 5A). In total, 83 P450 genes, 31 GST genes, 66 COE genes, 25 UDPGT genes, and 77 ABC transporter genes were annotated in *I. nitidus*. In addition, detoxification genes from the five gene families of four Scolytinae species (*I. nitidus*, *I. typographus*, *D. ponderosae*, and *Hypothenemus hampei*) and *Tribolium castaneum* were selected to construct the phylogenetic trees (Figures 5B and S4–S7).

Dominant symbiotic fungus, *Ophiostoma bicolor* affects gene expression in *I. nitidus*

The bioassay results showed that *I. nitidus* preferred substrates with *O. bicolor*-colonized side (Figures 6A and 6B). In the comparative transcriptome experiment, the beetles ate and tunneled in the *O. bicolor*-colonized or fungus-free substrates. On the *O. bicolor*-colonized substrates, beetles usually fed on some aerial mycelium and then started to tunnel next to fungal agar plugs or the sites where the mycelium flourishes (Figure 6C). In contrast, on fungus-free substrates, the sites of beetles starting to tunnel are random (Figure 6D). Based on DEG analysis of the transcriptome data from *I. nitidus* in the four treatments, we detected 111 upregulated genes and 225 downregulated genes in the comparison between GGQM (beetles that live in an *O. bicolor*-colonized substrate at high altitudes) and GGQA (beetles that live in a fungus-free substrate at high altitudes), and 193 upregulated genes and 73 downregulated genes in the comparison between DGQM (beetles that live in an *O. bicolor*-colonized substrate at low altitudes) and DGQA (beetles that live in a fungus-free substrate at low altitudes), respectively (Figure S8). At high altitudes, compared with GGQA, the function of upregulated genes of GGQM was enriched in the GO terms related to DNA damage repair and energy metabolism, as well as homologous recombination KEGG pathways (Figure 6E; Tables S13 and S14).

Table 5. Statistics for the noncoding RNAs

Type		Copy number	Average length (bp)	Total length (bp)
rRNA	rRNA	298	558.40	166,402
	18S	12	1,179.42	14,153
	28S	8	3,389.38	27,115
	5.8S	5	153.40	767
	5S	141	116.95	16,490
snRNA	snRNA	45	100.38	4,517
	CD-box	8	213.50	1,708
	HACA-box	1	192.00	192
	splicing	40	143.03	5,721
miRNA		60	27.30	1,638
tRNA		233	74.41	17,337

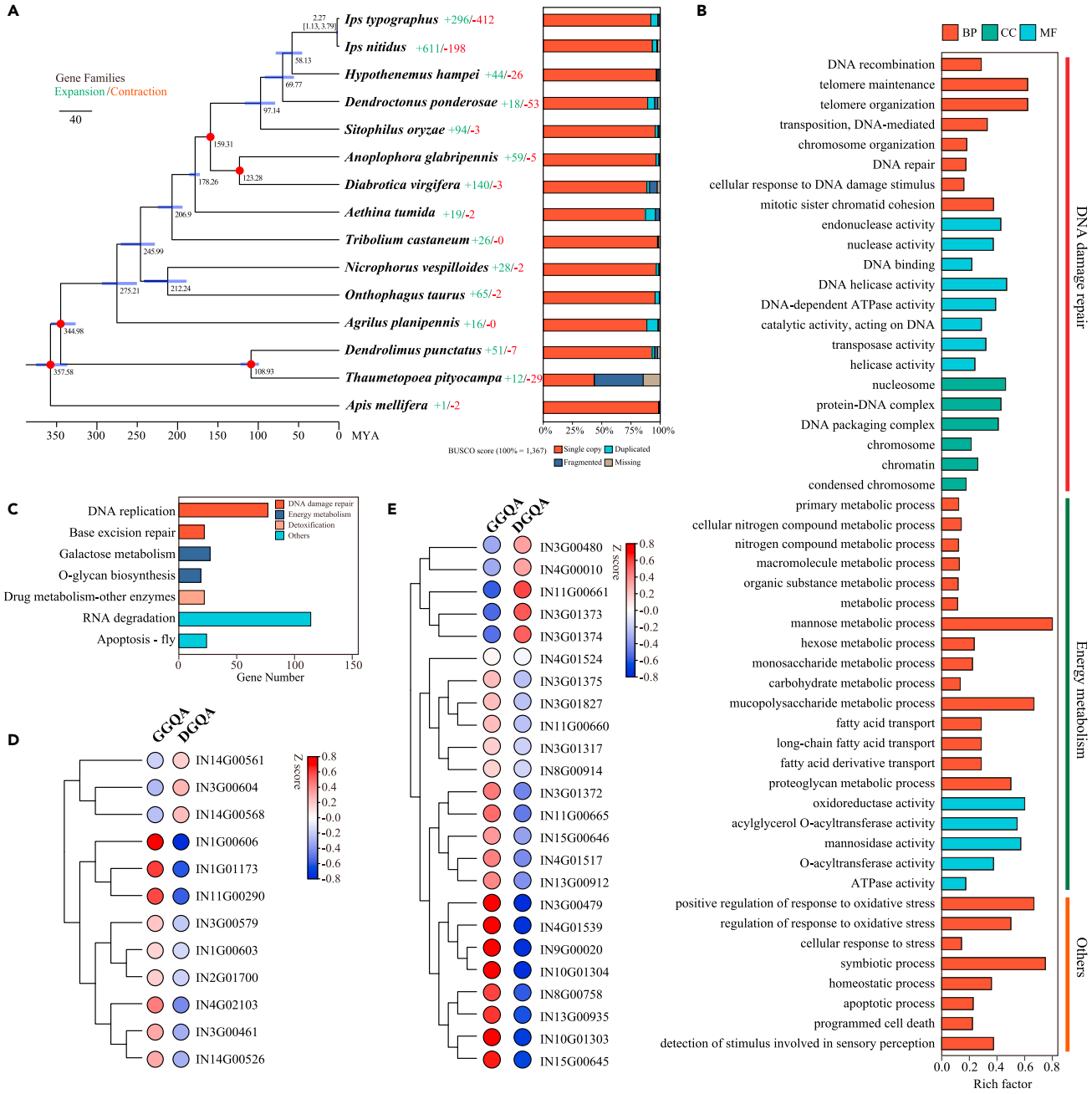


Figure 3. Gene family evolution between genomes of *I. nitidus* and 14 other species

(A) Genome evolution of *I. nitidus*. Divergence times and the 95% confidence intervals of the divergence time are indicated at the nodes.

(B) Gene ontology (GO) enrichment analysis of *I. nitidus* expansion gene family involving DNA damage repair, energy metabolism, and other categories that may be related to hypoxia adaptation. BP, biological process; CC, cellular component; MF, molecular function.

(C) KEGG enrichment analysis of *I. nitidus* expansion gene family.

(D) Heatmaps of gene expression involving DNA replication pathways in *I. nitidus* under high-altitude hypoxia (GGQA) than low-altitude normoxia (DGQA). GGQA: fed and tunneled in fungus-free semi-natural spruce bark substrates at high altitudes; DGQA: fed and tunneled in fungus-free semi-natural spruce bark substrates at low altitudes.

(E) Heatmaps of gene expression involving galactose metabolism pathways in *I. nitidus* under high-altitude hypoxia (GGQA) than low-altitude normoxia (DGQA). GGQA: fed and tunneled in fungus-free semi-natural spruce bark substrates at high altitudes; DGQA: fed and tunneled in fungus-free semi-natural spruce bark substrates at low altitudes.

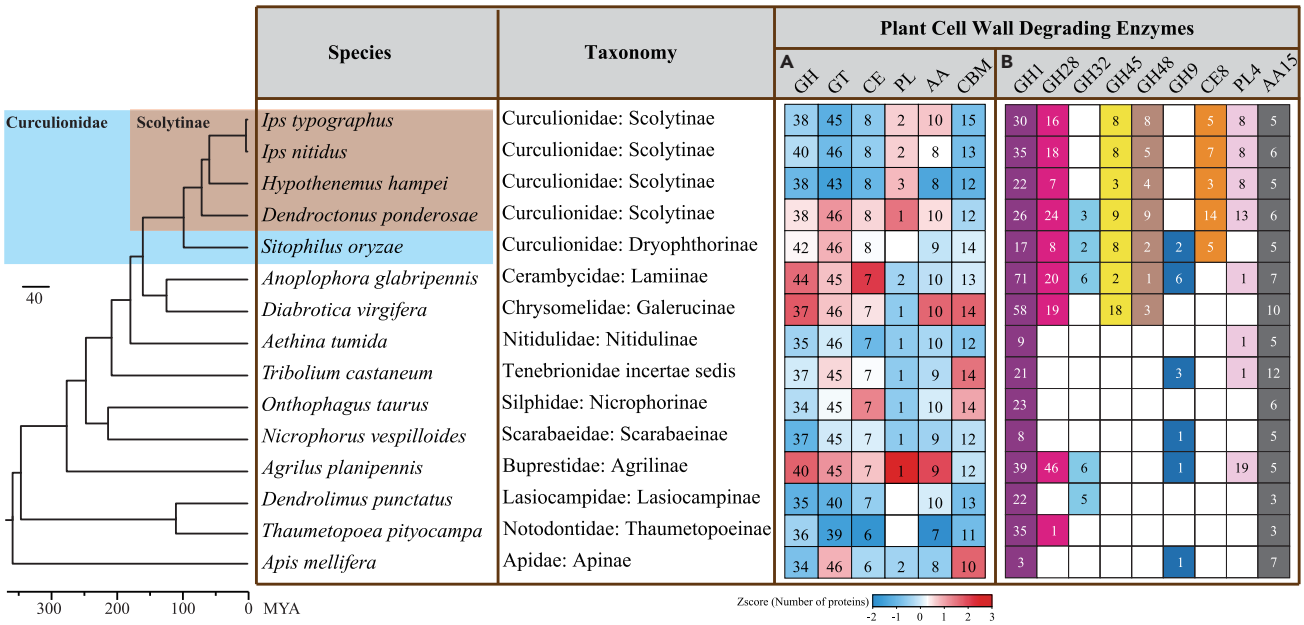


Figure 4. Plant cell wall-degrading enzymatic annotation of *I. nitidus* and 14 other species

(A) Numbers in the heatmap indicate the number of PCWDE families; colors represent the relative abundance of PCWDEs in six families, respectively.

(B) Numbers represent the number of PCWDEs; colors represent the presence or absence of the enzyme in the graph of selected representative nine PCWDE families.

However, compared with the two treatments at low altitudes (DGQM vs. DGQA), the upregulated genes were not involved in the above functions. Furthermore, the function of downregulated genes (GGQM vs. GGQA; DGQM vs. DGQA) was highly correlated with transmembrane transport-related at both high and low altitudes (Tables S13 and S14). A subsequent analysis of the genes encoding 77 ABC transporters in *I. nitidus* found that the beetles living in a fungus-colonized substrate had lower expression of most of these genes than the beetles living in fungus-free substrates (Figure 6F). These findings suggested that *O. bicolor* could affect the expression of genes that may be related to hypoxia adaptation and detoxification in beetles.

DISCUSSION

Although genome studies are common in insects, only a handful of forest pest genomes have been published. Chromosome-level assembled insect genomes are still rare. Considering the importance of genome studies in economically important forest pests, the current study represents the second genome from the *Ips* bark beetle, and the first chromosome-level assembled genome in *Ips*. The endurance of *I. nitidus* in harsh environments compared to its sister species makes the genome study more intriguing from an eco-evolutionary perspective.

Significant expansion and contraction of gene families are suggested to be closely related to the adaptive divergence of related species.[4,93,94] *Ips typographus* is a sister species to *I. nitidus*, which is only distributed at low altitudes and is equally damaging the spruce. Therefore, we analyzed functional enrichment and compared their extended gene families. The GO and KEGG enrichment analysis of expanded gene families and positively selected genes showed that *I. nitidus* had enriched functions involved in DNA damage repair and energy metabolism (Figures 3B and 3C; Tables S6, S7, and S10). Such enrichments were lacking in *I. typographus*. There were 42 GO categories (including 823 genes) of *I. nitidus* that were involved in DNA damage repair and energy metabolism (Figure 3B; Table S6), whereas *I. typographus* shared only eight (Table S15). We further compared set of genes contained in each of the previously described eight shared GO categories, and found that *I. nitidus* has 394 more genes than *I. typographus* (774 genes vs. 380 genes). Therefore, compared with *I. typographus*, *I. nitidus* exhibited more robust gene family expansions in the previous two functions involved in environmental adaptation in many high-altitude organisms. High-altitude organisms exposed to intense ultraviolet radiation and hypoxic conditions are prone to severe DNA damage.[95,96] Therefore, DNA damage repair is the key to their survival. Positive selection and expansion of genes involved in DNA repair are common in studies on the environmental adaptability of plateau flora and fauna, such as the Tibetan antelope,[4] Tibetan wild boars,[5] snub-nosed monkeys,[97] ectothermic snakes,[10] speckled teal,[95] *Crucihimalaya himalaica*,[94] and Tibetan semi-wild wheat.[98] In high-altitude vertebrates, genes that provide energy primarily function to cope with hypoxia and severe coldness. Positive selection and expansion of gene families related to energy metabolism are associated with hypoxic and frigid adaptation in different species.[4,5,7,99,100] Compared with vertebrates, there are few studies on the adaptation of insects to high-altitude hypoxia. Transcriptome analysis of Tibetan *Gynaephora*[101] and genomic analysis of Tibetan migratory locusts[13] found positively selected signals involving genes enriched in energy metabolism, which is consistent with our results (Table S10). Not only high-altitude animals but some aquatic insects also have to deal with hypoxia. *Aquatica*

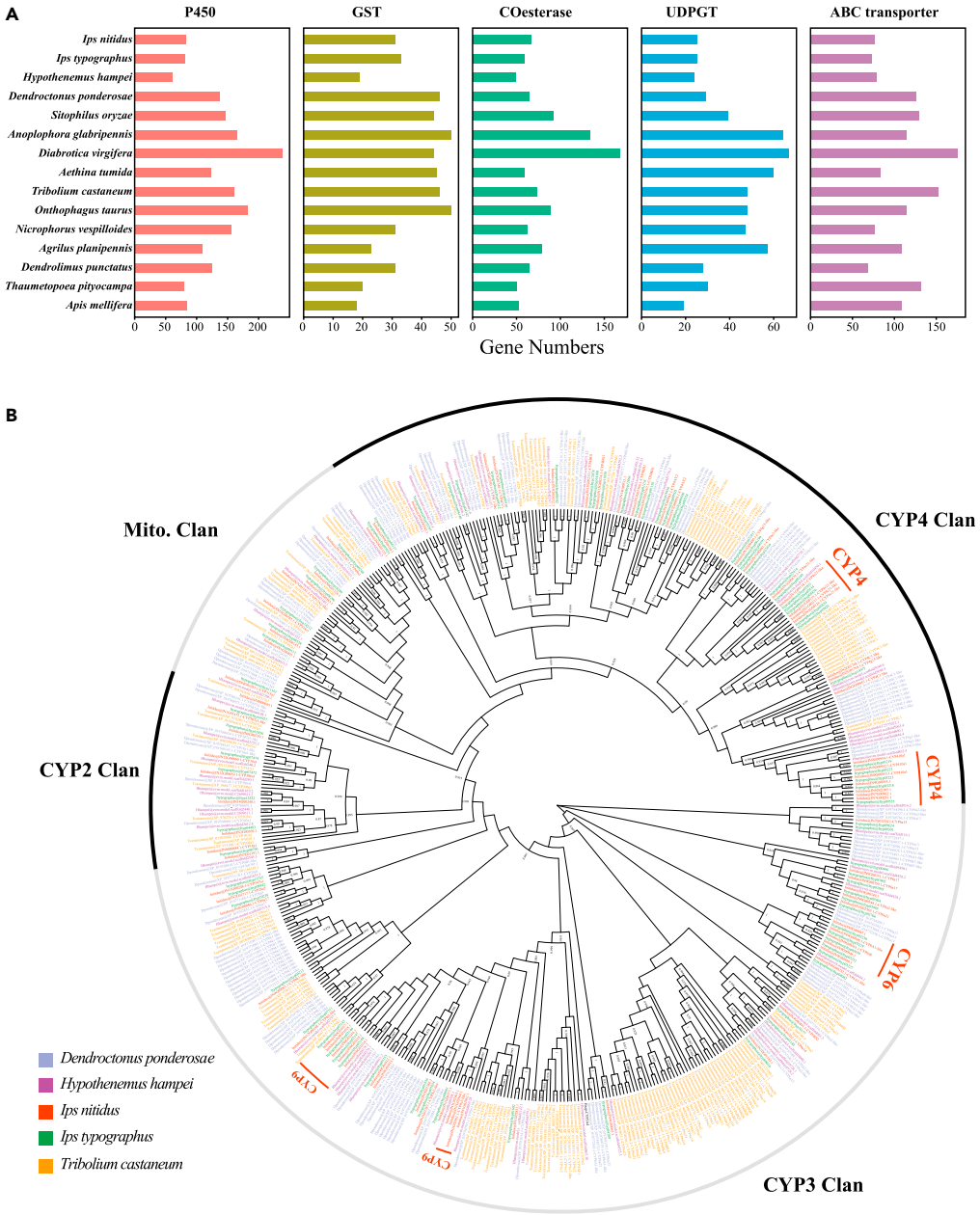


Figure 5. Gene family associated with detoxification

(A) Comparison of the number of genes in five detoxification-related gene families of *I. nitidus* and 14 other species.

(B) Phylogenetic analysis of P450s from *I. nitidus*, *I. typographus*, *H. hampei*, *D. ponderosae*, and *T. castaneum*. Red arcs indicate the expansion of the *I. nitidus*. More than 80% of the node support is marked on the node.

leii is a larval aquatic firefly (Coleoptera: Lampyridae). By comparing the transcriptome of this beetle with that of another entirely terrestrial firefly (*Lychnuris praetexta*), it is found that shared genes under positive selection are mainly involved in energy metabolism. A similar result was obtained through functional analysis with differentially expressed genes in larvae and adults.[102] Therefore, metabolic adaptation may be a crucial and conserved adaptive feature of the insect response to hypoxia. However, such claims and our current findings need further experimental corroboration.

As the most diverse insect order, beetles (Coleoptera) have been an intriguing research topic for decades.[47,103] Beetles have multiple PCWDEs, some of which are also present in animals (such as cellulases GH1 and GH9), while other cellulases (such as GH45 and GH48) and a pectinase (such as GH28) are microbially encoded PCWDEs derived from horizontal gene transfer. The adaptive radiation of

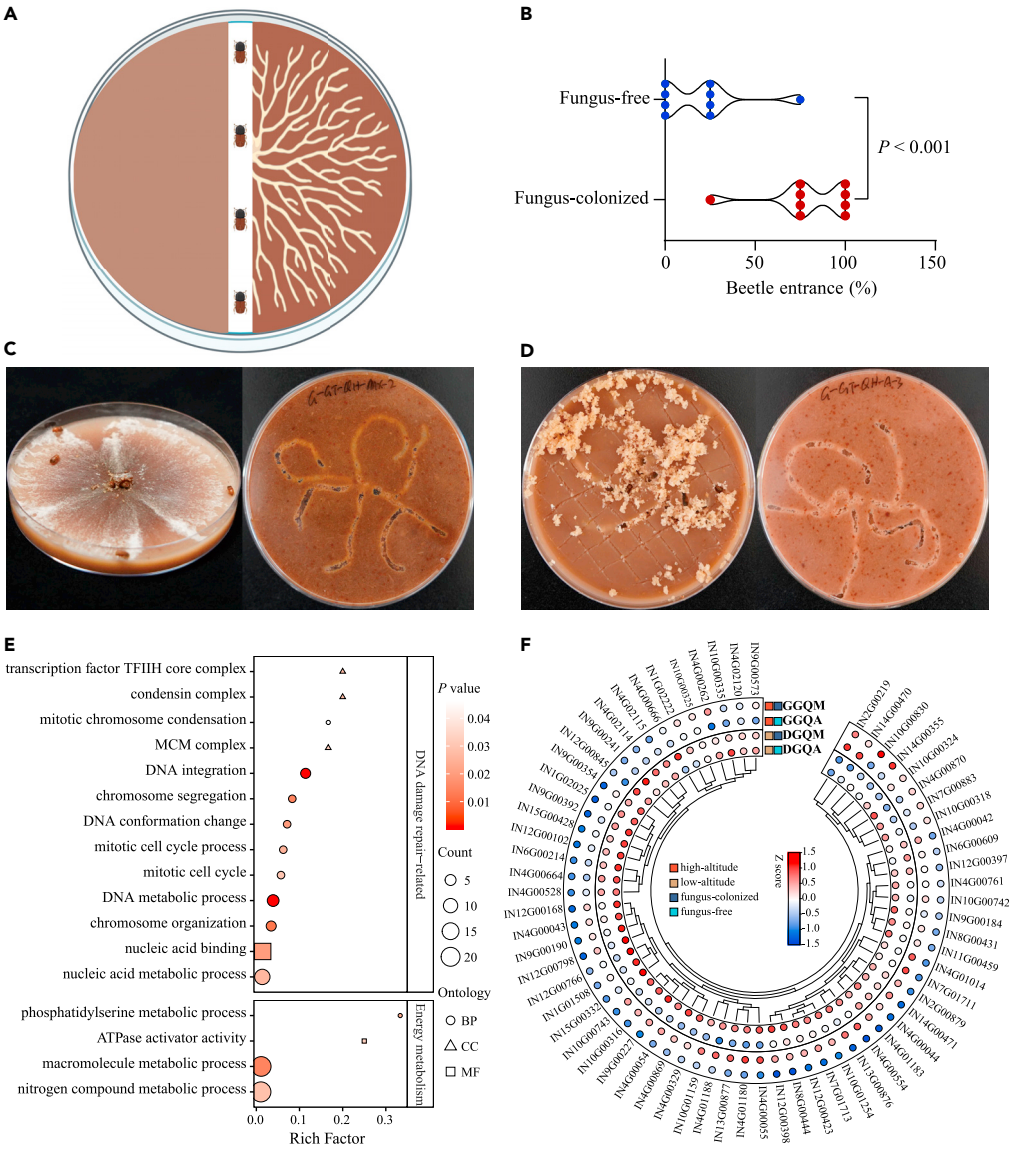


Figure 6. Selection preference of immature adult *Ips nitidus* for fungus-free or fungus-colonized semi-natural spruce bark substrates and functional enrichment of DEGs of *I. nitidus* feeding and tunneling in different substrates

(A) Choice arena consisted of two semicircles of substrate separated by a 5 mm wide strip of sterile 4% agar. Left side: fungus-free semi-natural spruce bark substrates. Right side: fungus-colonized semi-natural spruce bark substrates. Created with BioRender.com.

(B) The number of beetles entering different substrates was registered after 24 h.

(C) Beetles fed and tunneled in *O. bicolor*-colonized semi-natural spruce bark substrates.

(D) Beetles fed and tunneled in fungus-free semi-natural spruce bark substrates.

(E) Gene ontology (GO) enrichment analysis of upregulated genes in *I. nitidus* fed and tunneled in *O. bicolor*-colonized semi-natural spruce bark substrates than in fungus-free semi-natural spruce bark substrates at high altitude.

(F) Heatmaps of gene expression involving ABC transporters in *I. nitidus*. GGQM: fed and tunneled in *O. bicolor*-colonized semi-natural spruce bark substrates at high altitudes; GGQA: fed and tunneled in fungus-free semi-natural spruce bark substrates at high altitudes; DGQM: fed and tunneled in *O. bicolor*-colonized semi-natural spruce bark substrates at low altitudes; DGQA: fed and tunneled in fungus-free semi-natural spruce bark substrates at low altitudes.

herbivorous beetles after obtaining these PCWDEs is one of the fundamental reasons for beetle diversification.[48] The hypothesis that insect digestion of cellulose depends solely on gut microbes reformed when endogenous cellulase genes (GH1 and GH9) were found in termites.[104–107] GH1 and GH9 are thought to be ubiquitous in beetles. However, in Scolytinae, GH9 is lacking,[48] at least in the studies of genomes of *D. ponderosae*, *H. hampei*, and *I. typographus* that have been published so far and now in *I. nitidus* (Figure 4). In contrast, GH28, GH45, and GH48 were abundant in Scolytinae (Figure 4). GH32 invertases catalyze the conversion of sucrose to glucose and fructose, and they are

thought to play an important role in the evolution of some insects, such as Coleoptera and Lepidoptera.[48,108] However, it is not ubiquitous in beetles,[48] and in this study, it was only annotated in the genomes of *Agrilus planipennis*, *An. glabripennis*, *D. ponderosae*, and *S. oryzae* (Figure 4). GTs are indispensable multifaceted enzymatic regulators of insects, which play a crucial role in every insect's survival, growth, and development.[109] A study on silkworms found that a GT (quercetin 5-*O*-glucosyltransferase) produced cocoons with UV-shielding properties.[110] In addition, many insect GTs play crucial roles in detoxification, chemosensation, and stress responses.[109] We found that the total number of GT families was almost the same (between 39 and 46) among 15 tested insects but the total number of proteins varied widely (between 179 and 350) (Tables S11 and S12), therefore their functionality must be proven. Pectin methylesterase (CE8) is necessary to degrade the pectin main chain[111] but is only found in a few beetles,[48] which implies that many beetles rely on symbionts for this process. However, in our study, CE8 was found only in Curculionidae (Figure 4), which were known to have mutualistic relationships with microorganisms.[112] In addition, we annotated pectinase PL4 in *I. nitidus*, which is not present in most beetles yet is prevalent in Curculionidae genomes, such as *D. ponderosae*, *H. hampei*, and *I. typographus* (Figure 4). This suggests that Curculionidae beetles can degrade pectin independently without relying on symbionts. Lytic polysaccharide monooxygenases (LPMOs) are enzymes mainly found in microorganisms and viruses. However, the LPMO, AA15, is ubiquitous in insect genomes and may play an important role in insect development, food degradation, and overcoming host defenses.[113,114] Most insects have no more than five AA15 proteins,[114] but *I. nitidus* has six (Figure 4).

For conifer-feeding insects, the fundamental challenge is to cope with the formidable defenses of conifers.[53] Thus, conifer specialists are expected to have numerous genes involved in detoxifying monoterpenes and other entomotoxic compounds of conifer origin. However, we found that they do not exhibit enrichment in the detoxification gene family compared to other non-coniferous feeding species (Figure 5A). Conifer-feeding beetles may depend on the smaller but specialized P450s subfamilies to detoxify the hostile chemical defenses of the host.[35] We found significant differences in the number of P450 genes among the three conifer-feeding beetles. A total of 83 P450s annotated in the *I. nitidus* (spruce feeding) genome were about the same as the 81 identified in *I. typographus* (spruce feeding) and less than the 137 identified in *D. ponderosae* (pine feeding) (Figure 5A). Powell et al.[35] emphasized three factors, such as a mass attack, assistance from fungal symbionts, and the less inherent defenses of the host Norway spruce, to explain the success of *I. typographus* against conifer defenses, even with much fewer P450 genes than *D. ponderosae*. For spruce feeding beetles, P450 genes in *I. nitidus* are almost as numerous as that in *I. typographus*, suggesting that they are equally capable of detoxifying host chemical defenses or that the host plant may determine their ability to detoxify. Keeling et al.[115] saw "blooms" (lineage-specific expansions) in the CYP4, CYP6, and CYP9 in *D. ponderosae* and suggested specific P450 family expansions within different beetle species as part of their adaptations to different environments. Three CYPs blooms mentioned previously were also observed in *I. nitidus* and *I. typographus* (Figure 5B). This similar pattern suggests that these three CYPs may be critical to adapting the chemical defenses of conifer hosts. For the other four detoxification gene families, except ABC transporters and GSTs (Figures S4 and S5), 8 and 7 gene blooms were found in COEs (Figure S6) and UDPGTs (Figure S7), respectively. The function of these genes can be evaluated to understand better the molecular mechanism underlying *I. nitidus* detoxification of host chemical defense.

Metabolism of host chemical defenses (such as terpenoids and phenolics) by symbiotic fungi is fundamental for maintaining the beetle-fungus symbiosis.[41,71] Some ophiostomatoid fungi can degrade terpenoids and phenolics,[80,116] especially virulent associates. For example, *Endoconidiophora polonica* can alter phenolics into carbon sources for their growth.[81] However, the most consistent associates of bark beetles tend to be nonpathogenic or weak pathogens.[75] Therefore, we choose *O. bicolor*, the dominant associate of *I. nitidus*, to study symbiosis. We observed that many genes associated with detoxification were downregulated in *I. nitidus* fed and tunneled in *O. bicolor*-colonized spruce bark substrates compared with fungus-free substrates (Figure 6F). Such selective fine-tuning in the expression of the crucial detoxifying arsenal directly demonstrates that fungal symbionts assist vector beetles in detoxifying host chemical defenses and aid in minimizing the energy budget for the expression of detoxification machinery. Interestingly, the upregulated genes in fungus-feeding beetles at high-altitude treatments were mainly associated with hypoxia adaptation (Figure 6E; Tables S13 and S14). Functions related to DNA damage repair and energy metabolism were consistent with those of the beetle's expanded gene families (Figures 3B and 3C; Tables S6 and S7). This pattern suggests that the fungal symbiont *O. bicolor* may be an auxiliary for *I. nitidus* to cope with high-altitude hypoxia. Although such speculations need further experimental ratification, they lend a new glimpse into the symbiosis of beetles and fungi.

In conclusion, we assembled a chromosome-level genome of the conifer-feeding bark beetle, *I. nitidus*. Phylogenetic inference showed that *I. nitidus* diverged from the common ancestor of *I. typographus* at approximately 2.27 mya, which was after the range measured with the estimated uplift time of the Qinghai-Tibet Plateau. The expansion of many gene families in the *I. nitidus* genome is proposed to be related to high-altitude hypoxia adaptation. Transcriptome evidence suggests that fungal symbiont *O. bicolor* may assist *I. nitidus* in better coping with high-altitude hypoxia and detoxifying host chemical defenses. However, such possibilities need to be validated functionally. This study provides a new perspective for studying symbiosis between insects and fungi. Nevertheless, the present study delivers genomic resources and interesting testable hypotheses for future studies on insect adaptation to extreme environments and symbiosis with fungi, especially in bark beetles. It may aid in developing a novel forest pest management strategy using RNAi-FPPs targeting some key functional genes.

Limitations of the study

We assembled a high-quality chromosome-level genome of *I. nitidus* in this study. However, despite a single colony of beetles was collected to minimize heterogenicity, the plausible polymorphism in our specific DNA sequences might still be present due to the polymorphic nature of starting material. In addition, although our findings provide testable hypotheses associated with high-altitude hypoxia adaptation and fungal symbiosis of *I. nitidus*, the downstream functional experiments are needed to unravel the underlying molecular mechanisms.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
- METHOD DETAILS
  - Genome sequencing
  - Genome assembly and evaluation
  - Genome annotation
  - Phylogenetic inference and comparative genomic analysis
  - Choice aassay with fungus-free or fungus-colonized semi-natural spruce bark substrates
  - Transcriptome sequencing and analysis
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.107793.

ACKNOWLEDGMENTS

This study was supported by the National Key R&D Program of China (project no. 2017YFD0600103) and the National Natural Science Foundation of China (project no. 32230071 and 32071769). A.R. is supported by grant 'EXTEMIT-K,' (no. CZ.02. 1.01/0.0/0.0/15 _003/0000433) and EVA 4.0 (no. CZ.02.1.01/0.0/0.0/16_019/0000803) financed by OP RDE and Excellent team Grant (2023-24) from FLD, CZU. We thank Jianning Liu and Xiangwen Hou from Shandong Kaiyuan Gene Technology Co., Ltd for their help in data analysis, and Prof. Zhen Zhang and Sufang Zhang for their help in data collection.

AUTHOR CONTRIBUTIONS

Q.L., H.L., X.Z., and Z.W. designed the study. Z.W., F.H., Y.L., and H.W. collected the samples. Q.L. and Z.W. performed the research and analyzed the data. Q.L., Z.W., and A.R. wrote the manuscript. The final manuscript was reviewed and approved by all authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: February 6, 2023
Revised: May 15, 2023
Accepted: August 29, 2023
Published: August 30, 2023

REFERENCES

1. Deng, T., Wu, F., Zhou, Z., and Su, T. (2020). Tibetan Plateau: An evolutionary junction for the history of modern biodiversity. Sci. China Earth Sci. *63*, 172–187. https://doi.org/10.1007/s11430-019-9507-5.
2. Herzschuh, U., Birks, H.J.B., Ni, J., Zhao, Y., Liu, H., Liu, X., and Grosse, G. (2010). Holocene land-cover changes on the Tibetan Plateau. Holocene *20*, 91–104. https://doi.org/10.1177/0959683609348882.
3. Simonson, T.S., Yang, Y., Huff, C.D., Yun, H., Qin, G., Witherspoon, D.J., Bai, Z., Lorenzo, F.R., Xing, J., Jorde, L.B., et al. (2010). Genetic evidence for high-altitude adaptation in Tibet. Science *329*, 72–75. https://doi.org/10.1126/science.1189406.
4. Ge, R.L., Cai, Q., Shen, Y.Y., San, A., Ma, L., Zhang, Y., Yi, X., Chen, Y., Yang, L., Huang, Y., et al. (2013). Draft genome sequence of the Tibetan antelope. Nat. Commun. *4*, 1858–1867. https://doi.org/10.1038/ncomms2860.
5. Li, M., Tian, S., Jin, L., Zhou, G., Li, Y., Zhang, Y., Wang, T., Yeung, C.K.L., Chen, L., Ma, J., et al. (2013). Genomic analyses identify distinct patterns of selection in domesticated pigs and Tibetan wild boars. Nat. Genet. *45*, 1431–1438. https://doi.org/10.1038/ng.2811.
6. Li, C., Wu, Y., Chen, B., Cai, Y., Guo, J., Leonard, A.S., Kalds, P., Zhou, S., Zhang, J., Zhou, P., et al. (2022). Markhor-derived introgression of a genomic region encompassing PAPSS2 confers high-altitude adaptability in Tibetan goats. Mol. Biol. Evol. *39*, msac253. https://doi.org/10.1093/molbev/msac253.
7. Qiu, Q., Zhang, G., Ma, T., Qian, W., Wang, J., Ye, Z., Cao, C., Hu, Q., Kim, J., Larkin, D.M., et al. (2012). The yak genome and adaptation to life at high altitude. Nat. Genet. *44*, 946–949. https://doi.org/10.1038/ng.2343.
8. Zhang, W., Fan, Z., Han, E., Hou, R., Zhang, L., Galaverni, M., Huang, J., Liu, H., Silva, P., Li, P., et al. (2014). Hypoxia adaptations in the grey wolf (*Canis lupus chanco*) from Qinghai-Tibet Plateau. PLoS Genet. *10*, e1004466. https://doi.org/10.1371/journal.pgen.1004466.
9. Qu, Y., Zhao, H., Han, N., Zhou, G., Song, G., Gao, B., Tian, S., Zhang, J., Zhang, R., Meng, X., et al. (2013). Ground tit genome reveals avian adaptation to living at high altitudes in the Tibetan plateau. Nat. Commun. *4*, 2071. https://doi.org/10.1038/ncomms3071.

10. Li, J.T., Gao, Y.D., Xie, L., Deng, C., Shi, P., Guan, M.L., Huang, S., Ren, J.L., Wu, D.D., Ding, L., et al. (2018). Comparative genomic investigation of high-elevation adaptation in ectothermic snakes. Proc. Natl. Acad. Sci. USA *115*, 8406–8411. https://doi.org/10.1073/pnas.1805348115.
11. Wang, G.D., Zhang, B.L., Zhou, W.W., Li, Y.X., Jin, J.Q., Shao, Y., Yang, H.C., Liu, Y.H., Yan, F., Chen, H.M., et al. (2018). Selection and environmental adaptation along a path to speciation in the Tibetan frog *Nanorana parkeri*. Proc. Natl. Acad. Sci. USA *115*, E5056–E5065. https://doi.org/10.1073/pnas.1716257115.
12. Xiao, S., Mou, Z., Fan, D., Zhou, H., Zou, M., Zou, Y., Zhou, C., Yang, R., Liu, J., Zhu, S., et al. (2020). Genome of tetraploid fish Schizothorax o'connori provides insights into early re-diploidization and high-altitude adaptation. iScience *23*, 101497. https://doi.org/10.1016/j.isci.2020.101497.
13. Ding, D., Liu, G., Hou, L., Gui, W., Chen, B., and Kang, L. (2018). Genetic variation in PTPN1 contributes to metabolic adaptation to high-altitude hypoxia in Tibetan migratory locusts. Nat. Commun. *9*, 4991. https://doi.org/10.1038/s41467-018-07529-8.
14. Misof, B., Liu, S., Meusemann, K., Peters, R.S., Donath, A., Mayer, C., Frandsen, P.B., Ware, J., Flouri, T., Beutel, R.G., et al. (2014). Phylogenomics resolves the timing and pattern of insect evolution. Science *346*, 763–767. https://doi.org/10.1126/science.1257570.
15. Biedermann, P.H.W., Müller, J., Grégoire, J.C., Gruppe, A., Hagge, J., Hammerbacher, A., Hofstetter, R.W., Kandasamy, D., Kolarik, M., Kostovcik, M., et al. (2019). Bark beetle population dynamics in the Anthropocene: challenges and solutions. Trends Ecol. Evol. *34*, 914–924. https://doi.org/10.1016/j.tree.2019.06.002.
16. Jakoby, O., Lischke, H., and Wermelinger, B. (2019). Climate change alters elevational phenology patterns of the European spruce bark beetle (*Ips typographus*). Global Change Biol. *25*, 4048–4063. https://doi.org/10.1111/gcb.14766.
17. Kurz, W.A., Dymond, C.C., Stinson, G., Rampley, G.J., Neilson, E.T., Carroll, A.L., Ebata, T., and Safranyik, L. (2008). Mountain pine beetle and forest carbon feedback to climate change. Nature *452*, 987–990. https://doi.org/10.1038/nature06777.
18. Logan, J.A., Régnière, J., and Powell, J.A. (2003). Assessing the impacts of global warming on forest pest dynamics. Front. Ecol. Environ. *1*, 130–137. https://doi.org/10.1890/1540-9295(2003)001[0130:ATIOGW]2.0.CO;2.
19. Pettit, J.M., Voelker, S.L., DeRose, R.J., and Burton, J.I. (2020). Spruce beetle outbreak was not driven by drought stress: Evidence from a tree-ring iso-demographic approach indicates temperatures were more important. Global Change Biol. *26*, 5829–5843. https://doi.org/10.1111/gcb.15274.
20. Dale, V.H., Joyce, L.A., McNulty, S., Neilson, R.P., Ayres, M.P., Flannigan, M.D., Hanson, P.j., Irland, L.C., Lugo, A.E., Peterson, C.J., et al. (2001). Climate change and forest disturbances. Bioscience *51*, 723–734. https://doi.org/10.1641/0006-3568(2001)051[0723:CCAFD]2.0.CO;2.
21. Grégoire, J.C., Raffa, K.F., and Lindgren, B.S. (2015). Chapter 15 - Economics and politics of bark beetles. In Bark Beetles, F.E. Vega and R.W. Hofstetter, eds. (Academic Press), pp. 585–613. https://doi.org/10.1016/B978-0-12-417156-5.00015-0.
22. KärvEmo, S., and Schroeder, L.M. (2010). A comparison of outbreak dynamics of the spruce bark beetle in Sweden and the mountain pine beetle in Canada (Curculionidae: Scolytinae). Entomol. Tidskr. *131*, 215–224.
23. Schelhaas, M.J., Nabuurs, G.J., and Schuck, A. (2003). Natural disturbances in the European forests in the 19th and 20th centuries. Global Change Biol. *9*, 1620–1633. https://doi.org/10.1046/j.1365-2486.2003.00684.x.
24. Seidl, R., Schelhaas, M.J., and Lexer, M.J. (2011). Unraveling the drivers of intensifying forest disturbance regimes in Europe. Global Change Biol. *17*, 2842–2852. https://doi.org/10.1111/j.1365-2486.2011.02452.x.
25. Cognato, A.I. (2015). Chapter 9. Biology, systematics, and evolution of *Ips*. In Bark Beetles, F.E. Vega and R.W. Hofstetter, eds. (Academic Press), pp. 351–370. https://doi.org/10.1016/B978-0-12-417156-5.00009-5.
26. Raffa, K.F., Andersson, M.N., and Schlyter, F. (2016). Host selection by bark beetles: playing the odds in a high-stakes game. Adv. Insect Physiol *50*, 1–74. https://doi.org/10.1016/bs.aiip.2016.02.001.
27. Zhang, Q.H., Cao, J., Hu, Y.z., Huang, Y.h., Lü, S.y., Wei, G.z., and Zhao, Y.f. (2004). Olfactory recognition and behavioural avoidance of angiosperm nonhost volatiles by conifer-inhabiting bark beetles. Agric. For. Entomol. *20*, 1–6. https://doi.org/10.1111/j.1461-9555.2004.00202.x.
28. Birgersson, G., Schlyter, F., Löfqvist, J., and Bergström, G. (1984). Quantitative variation of pheromone components in the spruce bark beetle *Ips typographus* from different attack phases. J. Chem. Ecol. *10*, 1029–1055. https://doi.org/10.1007/BF00987511.
29. Schlyter, F., Byers, J.A., and Löfqvist, J. (1987). Attraction to pheromone sources of different quantity, quality, and spacing: Density-regulation mechanisms in bark beetle *Ips typographus*. J. Chem. Ecol. *13*, 1503–1523. https://doi.org/10.1007/BF01012294.
30. Schlyter, F., and Birgersson, G. (1999). Forest Beetles. In Pheromones in Non-Lepidopteran Insects Associated with Agricultural Plants, J. Hardie and A.K. Minks, eds. (Oxford), pp. 113–148.
31. Hlásny, T., König, L., Krokene, P., Lindner, M., Montagné-Huck, C., Müller, J., Qin, H., Raffa, K.F., Schelhaas, M.J., Svoboda, M., et al. (2021). Bark beetle outbreaks in Europe: state of knowledge and ways forward for management. Curr. For. Rep. *7*, 138–165. https://doi.org/10.1007/s40725-021-00142-x.
32. Wang, Z.B., Chen, Y.Q., Wang, F.Y., Wang, H.H., Xu, H.Z., Li, X.P., and Song, L.W. (2013). Research on the application technology of aggregation pheromone of *Ips typographus* L. J Jilin For Sci Tech *42*, 24–26. https://doi.org/10.3969/j.issn.1005-7129.2013.03.008.
33. Yue, C.Y., Hou, Y.H., Alimu, M., and Sun, G.Q. (2014). Control and division of *Ips typographus* Linnaeus in natural forest of Aertai mountain in Xinjiang region. Hubei Agric. Sci. *53*, 4059–4062.
34. Furuta, K. (1989). A comparison of endemic and epidemic populations of the spruce beetle (*Ips typographus japonicus* Niijima) in Hokkaido. J. Appl. Entomol. *107*, 289–295. https://doi.org/10.1111/j.1439-0418.1989.tb00258.x.
35. Powell, D., Groβe-Wilde, E., Krokene, P., Roy, A., Chakraborty, A., Löfstedt, C., Vogel, H., Andersson, M.N., and Schlyter, F. (2021). A highly-contiguous genome assembly of the Eurasian spruce bark beetle, *Ips typographus*, provides insight into a major forest pest. Commun. Biol. *4*, 1–9. https://doi.org/10.1038/s42003-021-02602-3.
36. Wang, Z., Ma, X., Zhou, Q., Zheng, G., Xia, W., Zhang, Y., Wang, C., Jin, P., Lu, Q., and Zhang, X. (2021). Identification of *Ips* species (Coleoptera: Scolytinae) in China. Sci. Silvae Sin. *57*, 79–91.
37. Liu, L., Wu, J., Luo, Y.Q., Li, Z.Y., Wang, G.C., and Han, F.Z. (2008). Morphological and biological investigation of two pioneer *Ips* bark beetles in natural spruce forests in Qinghai Province, northwest China. For. Stud. China *36*, 19–25. https://doi.org/10.1007/s11632-008-0005-y.
38. Jakuš, R., Kalinová, B., Hoskovec, M., Knížek, M., Schlyter, F., Sun, J.H., and Zhang, Q.H. (2011). Outbreak of *Ips nitidus* and *Ips shangrila* in northeastern margin of the Tibetan Plateau. In 10th IUFRO Workshop of WP, *7*, pp. 20–23.
39. Yin, H.F., Huang, F.S., and Li, Z.L. (1984). Economic Insect Fauna of China (Fasc. 29) Coleoptera: Scolytidae (Science Press), pp. 126–136.
40. Liu, X., Serova, L., Kvetnanský, R., Sabban, E.L., Qin, D., and Ren, J. (2008). Response and dendroclimatic implications of δ13C in tree rings to increasing drought on the northeastern Tibetan Plateau. Ann. N. Y. Acad. Sci. *1148*, 1–28. https://doi.org/10.1029/2007JG000610.
41. Netherer, S., Kandasamy, D., Jirosová, A., Kalinová, B., Schebeck, M., and Schlyter, F. (2021). Interactions among Norway spruce, the bark beetle *Ips typographus* and its fungal symbionts in times of drought. J. Pest. Sci. *1–24*. https://doi.org/10.1007/s10340-021-01341-y.
42. Fu, H.E. (1983). The life habits of *Ips nitidus* and its control. J Beijing For Coll *4*, 30–38. https://doi.org/10.13332/j.1000-1522.1983.04.004.
43. Zhang, Q.H., Ma, J.H., Zhao, F.Y., Song, L.W., and Sun, J.H. (2009). Aggregation pheromone of the Qinghai spruce bark beetle, *Ips nitidus* Eggers. J. Chem. Ecol. *35*, 610–617. https://doi.org/10.1007/s10886-009-9634-4.
44. Zhou, J.X., Li, M.L., Li, H.H., and Miller, M.C. (1995). A Study on Luring Some Beetles of China by Using Pheromones of North American Beetles. J Northwest For Coll *10*, 74–80.
45. Fettig, C.J., and Hilszczański, J. (2015). Chapter 14 - Management strategies for bark beetles in conifer forests. In Bark Beetles, F.E. Vega and R.W. Hofstetter, eds. (Academic Press), pp. 555–584. https://doi.org/10.1016/B978-0-12-417156-5.00014-9.
46. Kenrick, P., and Crane, P.R. (1997). The origin and early evolution of plants on land. Nature *389*, 33–39. https://doi.org/10.1038/37918.
47. Farrell, B.D. (1998). "Inordinate fondness" explained: Why are there so many beetles?

Science *281*, 555–559. https://doi.org/10.1126/science.281.5376.555.
48. McKenna, D.D., Shin, S., Ahrens, D., Balke, M., Beza-Beza, C., Clarke, D.J., Donath, A., Escalona, H.E., Friedrich, F., Letsch, H., et al. (2019). The evolution and genomic basis of beetle diversity. Proc. Natl. Acad. Sci. USA *116*, 24729–24737. https://doi.org/10.1073/pnas.1909655116.
49. Després, L., David, J.-P., and Gallet, C. (2007). The evolutionary ecology of insect resistance to plant chemicals. Trends Ecol. Evol. *22*, 298–307. https://doi.org/10.1016/j.tree.2007.02.010.
50. Seppey, M., Ioannidis, P., Emerson, B.C., Pitteloud, C., Robinson-Rechavi, M., Roux, J., Escalona, H.E., McKenna, D.D., Misof, B., Shin, S., et al. (2019). Genomic signatures accompanying the dietary shift to phytophagy in polyphagan beetles. Genome Biol. *20*, 98. https://doi.org/10.1186/s13059-019-1704-5.
51. Seybold, S.J., Bohlmann, J., and Raffa, K.F. (2000). Biosynthesis of coniferophagous bark beetle pheromones and conifer isoprenoids: evolutionary perspective and synthesis. Can. Entomol. *132*, 697–753. https://doi.org/10.4039/Ent132697-6.
52. Celedon, J.M., and Bohlmann, J. (2019). Oleoresin defenses in conifers: chemical diversity, terpene synthases and limitations of oleoresin defence under climate change. New Phytol. *224*, 1444–1463. https://doi.org/10.1111/nph.15984.
53. Franceschi, V.R., Krokene, P., Christiansen, E., and Krekling, T. (2005). Anatomical and chemical defenses of conifer bark against bark beetles and other pests. New Phytol. *167*, 353–375. https://doi.org/10.1111/j.1469-8137.2005.01436.x.
54. Keeling, C.I., and Bohlmann, J. (2006). Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. New Phytol. *170*, 657–675. https://doi.org/10.1111/j.1469-8137.2006.01716.x.
55. Phillips, M.A., and Croteau, R.B. (1999). Resin-based defenses in conifers. Trends Plant Sci. *4*, 184–190. https://doi.org/10.1016/S1360-1385(99)01401-6.
56. Trapp, S., and Croteau, R. (2001). Defensive resin biosynthesis in conifers. Annu. Rev. Plant Biol. *52*, 689–724. https://doi.org/10.1146/annurev.arplant.52.1.689.
57. Keeling, C.I., Tittiger, C., MacLean, M., and Blomquist, G.J. (2020). Pheromone production in bark beetles. In Insect Pheromone Biochemistry and Molecular Biology, G.J. Blomquist and R.G. Vogt, eds. (Academic Press), pp. 123–162. https://doi.org/10.1016/B978-0-12-819628-1.00004-3.
58. Andersson, M.N., Grosse-Wilde, E., Keeling, C.I., Bengtsson, J.M., Yuen, M.M.S., Li, M., Hillbur, Y., Bohlmann, J., Hansson, B.S., and Schlyter, F. (2013). Antennal transcriptome analysis of the chemosensory gene families in the tree killing bark beetles, *Ips typographus* and *Dendroctonus ponderosae* (Coleoptera: Curculionidae: Scolytinae). BMC Genom. *14*, 198–216. https://doi.org/10.1186/1471-2164-14-198.
59. Hou, X.Q., Yuvaraj, J.K., Roberts, R.E., Zhang, D.D., Unelius, C.R., Löfstedt, C., and Andersson, M.N. (2021). Functional evolution of a bark beetle odorant receptor clade detecting monoterpenoids of different ecological origins. Mol. Biol. Evol. *38*, 4934–4947. https://doi.org/10.1093/molbev/msab218.
60. Ramakrishnan, R., Hradecký, J., Roy, A., Kalinová, B., Mendezes, R.C., Synek, J., Bláha, J., Svatoš, A., and Jirošová, A. (2022). Metabolomics and transcriptomics of pheromone biosynthesis in an aggressive forest pest *Ips typographus*. Insect Biochem. Mol. Biol. *140*, 103680. https://doi.org/10.1016/j.ibmb.2021.103680.
61. Joga, M.R., Mogilicherla, K., Smagghe, G., and Roy, A. (2021). RNA Interference-Based Forest Protection Products (FPPs) Against Wood-Boring Coleopterans: Hope or Hype? Front. Plant Sci. *12*, 733608. https://doi.org/10.3389/fpls.2021.733608.
62. Chakraborty, A., Ashraf, M.Z., Modlinger, R., Synek, J., Schlyter, F., and Roy, A. (2020). Unravelling the gut bacteriome of *Ips* (Coleoptera: Curculionidae: Scolytinae): Identifying core bacterial assemblage and their ecological relevance. Sci. Rep. *10*, 18572–18617. https://doi.org/10.1038/s41598-020-75203-5.
63. Chakraborty, A., and Roy, A. (2021). Microbial Influence on Plant–Insect Interaction. In Plant-Pest Interactions: From Molecular Mechanisms to Chemical Ecology, A. Singh and I.K. Singh, eds. (Springer), pp. 337–363. https://doi.org/10.1007/978-981-15-2467-7_14.
64. Liu, F., Ye, F., Cheng, C., Kang, Z., Kou, H., and Sun, J. (2022). Symbiotic microbes aid host adaptation by metabolizing a deterrent host pine carbohydrate d-pinitol in a beetle-fungus invasive complex. Sci. Adv. *8*, eadd5051. https://doi.org/10.1126/sciadv.add5051.
65. Salem, H., and Kaltenpoth, M. (2022). Beetle–bacterial symbioses: endless forms most functional. Annu. Rev. Entomol. *67*, 201–219. https://doi.org/10.1146/annurev-ento-061421-063433.
66. Schmidt, K., and Engel, P. (2021). Mechanisms underlying gut microbiota–host interactions in insects. J. Exp. Bot. *224*, jeb207696. https://doi.org/10.1242/jeb.207696.
67. Joseph, R., and Keyhani, N.O. (2021). Fungal mutualisms and pathosystems: life and death in the ambrosia beetle mycangia. Appl. Microbiol. Biotechnol. *105*, 3393–3410. https://doi.org/10.1007/s00253-021-11268-0.
68. Nagendran, S., Agrawal, S.S., and Abraham, S. (2021). Symbiotic Harmony Between Insects and Fungi: A Mutualistic Approach. In Symbiotic Soil Microorganisms, N. Shrivastava and A. Varma, eds. (Springer), pp. 269–288. https://doi.org/10.1007/978-3-030-51916-2_17.
69. Hofstetter, R.W., Dinkins-Bookwalter, J., Davis, T.S., and Klepzig, K.D. (2015). Symbiotic associations of bark beetles. In Bark Beetles, F.E. Vega and R.W. Hofstetter, eds. (Academic Press), pp. 209–245. https://doi.org/10.1016/B978-0-12-417156-5.00006-X.
70. Kandasamy, D., Zaman, R., Nakamura, Y., Zhao, T., Hartmann, H., Andersson, M.N., Hammerbacher, A., and Gershenzon, J. (2023). Conifer-killing bark beetles locate fungal symbionts by detecting volatile fungal metabolites of host tree resin monoterpenes. PLoS Biol. *21*, e3001887. https://doi.org/10.1371/journal.pbio.3001887.
71. Zaman, R., May, C., Ullah, A., and Erbilgin, N. (2023). Bark Beetles Utilize Ophiostomatoid Fungi to Circumvent Host Tree Defenses. Metabolites *13*, 239. https://doi.org/10.3390/metabo13020239.
72. Chakraborty, A., Modlinger, R., Ashraf, M.Z., Synek, J., Schlyter, F., and Roy, A. (2020). Core mycobiome and their ecological relevance in the gut of five *Ips* bark beetles (Coleoptera: Curculionidae: Scolytinae). Front. Microbiol. *11*, 568853. https://doi.org/10.3389/fmicb.2020.568853.
73. de Beer, Z.W., and Wingfield, M.J. (2013). Emerging lineages in the *Ophiostomatales*. In The Ophiostomatoid fungi: expanding frontiers, K.A. Seifert, Z.W. De Beer, and M.J. Wingfield, eds. (CBS-KNAW Fungal Biodiversity Centre), pp. 21–46.
74. de Beer, Z.W., Duong, T.A., Barnes, I., Wingfield, B.D., and Wingfield, M.J. (2014). Redefining *Ceratocystis* and allied genera. Stud. Mycol. *79*, 187–219. https://doi.org/10.1016/j.simyco.2014.10.001.
75. Six, D.L., and Wingfield, M.J. (2011). The role of phytopathogenicity in bark beetle–fungus symbioses: a challenge to the classic paradigm. Annu. Rev. Entomol. *56*, 255–272. https://doi.org/10.1146/annurev-ento-120709-144839.
76. Bracewell, R.R., and Six, D.L. (2015). Experimental evidence of bark beetle adaptation to a fungal symbiont. Ecol. Evol. *5*, 5109–5119. https://doi.org/10.1002/ece3.1772.
77. Davis, T.S., Stewart, J.E., Mann, A., Bradley, C., and Hofstetter, R.W. (2019). Evidence for multiple ecological roles of *Leptographium abietinum*, a symbiotic fungus associated with the North American spruce beetle. Fungal Ecol. *38*, 62–70. https://doi.org/10.1016/j.funeco.2018.04.008.
78. Guevara-Rozo, S., Hussain, A., Cale, J.A., Klutsch, J.G., Rajabzadeh, R., and Erbilgin, N. (2020). Nitrogen and Ergosterol Concentrations Varied in Live Jack Pine Phloem Following Inoculations with Fungal Associates of Mountain Pine Beetle. Front. Microbiol. *11*, 1703. https://doi.org/10.3389/fmicb.2020.01703.
79. Six, D.L. (2013). The bark beetle holobiont: why microbes matter. J. Chem. Ecol. *39*, 989–1002. https://doi.org/10.1007/s10886-013-0318-8.
80. DiGuistini, S., Wang, Y., Liao, N.Y., Taylor, G., Tanguay, P., Feau, N., Henrissat, B., Chan, S.K., Hesse-Orce, U., Alamouti, S.M., et al. (2011). Genome and transcriptome analyses of the mountain pine beetle-fungal symbiont *Grosmannia clavigera*, a lodgepole pine pathogen. Proc. Natl. Acad. Sci. USA *108*, 2504–2509. https://doi.org/10.1073/pnas.1011289108.
81. Wadke, N., Kandasamy, D., Vogel, H., Lah, L., Wingfield, B.D., Paetz, C., Wright, L.P., Gershenzon, J., and Hammerbacher, A. (2016). The bark-beetle-associated fungus, *Endoconidiophora polonica*, utilizes the phenolic defense compounds of its host as a carbon source. Plant Physiol. *171*, 914–931. https://doi.org/10.1104/pp.15.01916.
82. Cale, J.A., Ding, R., Wang, F., Rajabzadeh, R., and Erbilgin, N. (2019). Ophiostomatoid fungi can emit the bark beetle pheromone verbenone and other semiochemicals in media amended with various pine chemicals

and beetle-released compounds. Fungal Ecol. *39*, 285–295. https://doi.org/10.1016/j.funeco.2019.01.003.

83. Kandasamy, D., Gershenzon, J., Andersson, M.N., and Hammerbacher, A. (2019). Volatile organic compounds influence the interaction of the Eurasian spruce bark beetle (*Ips typographus*) with its fungal symbionts. ISME J. *13*, 1788–1800. https://doi.org/10.1038/s41396-019-0390-3.
84. Zhao, T., Axelsson, K., Krokene, P., and Borg-Karlson, A.K. (2015). Fungal symbionts of the spruce bark beetle synthesize the beetle aggregation pheromone 2-methyl-3-buten-2-ol. J. Chem. Ecol. *41*, 848–852. https://doi.org/10.1007/s10886-015-0617-3.
85. Zhao, T., Ganji, S., Schiebe, C., Bohman, B., Weinstein, P., Krokene, P., Borg-Karlson, A.K., and Unelius, C.R. (2019). Convergent evolution of semiochemicals across Kingdoms: bark beetles and their fungal symbionts. ISME J. *13*, 1535–1545. https://doi.org/10.1038/s41396-019-0370-7.
86. Davis, T.S., Hofstetter, R.W., Foster, J.T., Foote, N.E., and Keim, P. (2011). Interactions between the yeast *Ogataea pini* and filamentous fungi associated with the western pine beetle. Microb. Ecol. *61*, 626–634. https://doi.org/10.1007/s00248-010-9773-8.
87. Skelton, J., Jusino, M.A., Carlson, P.S., Smith, K., Banik, M.T., Lindner, D.L., Palmer, J.M., and Hulcr, J. (2019). Relationships among wood-boring beetles, fungi, and the decomposition of forest biomass. Mol. Ecol. *28*, 4971–4986. https://doi.org/10.1111/mec.15263.
88. Sahara, K., Marec, F., and Traut, W. (1999). TTAGG telomeric repeats in chromosomes of some insects and other arthropods. Chromosome Res. *7*, 449–460.
89. Smith, S.G. (1953). Chromosome numbers of Coleoptera. II. Heredity *7*, 31–48.
90. Wang, C., Dai, J., Zhao, X., Li, Y., Graham, S.A., He, D., Ran, B., and Meng, J. (2014). Outward-growth of the Tibetan Plateau during the Cenozoic: A review. Tectonophysics *621*, 1–43. https://doi.org/10.1016/j.tecto.2014.01.036.
91. Chimpanzee Sequencing and Analysis Consortium, Hillier, L., Eichler, E., Zody, M., Jaffe, D., Yang, S.P., Enard, W., Hellmann, I., Lindblad-Toh, K., Altheide, T.K., et al. (2005). Initial sequence of the chimpanzee genome and comparison with the human genome. Nature *437*, 69–87. https://doi.org/10.1038/nature04072.
92. Sudmant, P.H., Kitzman, J.O., Antonacci, F., Alkan, C., Malig, M., Tsalenko, A., Sampas, N., Bruhn, L., Shendure, J.; 1000 Genomes Project, and Eichler, E.E. (2010). Diversity of human copy number variation and multicopy genes. Science *330*, 641–646. https://doi.org/10.1126/science.1197005.
93. Wu, N., Zhang, S., Li, X., Cao, Y., Liu, X., Wang, Q., Liu, Q., Liu, H., Hu, X., Zhou, X.J., et al. (2019). Fall webworm genomes yield insights into rapid adaptation of invasive species. Nat. Ecol. Evol. *3*, 105–115. https://doi.org/10.1038/s41559-018-0746-5.
94. Zhang, T., Qiao, Q., Novikova, P.Y., Wang, Q., Yue, J., Guan, Y., Ming, S., Liu, T., De, J., Liu, Y., et al. (2019). Genome of *Crucihimalaya himalaica*, a close relative of *Arabidopsis*, shows ecological adaptation to high altitude. Proc. Natl. Acad. Sci. USA *116*, 7137–7146. https://doi.org/10.1073/pnas.1817580116.
95. Graham, A.M., Lavretsky, P., Muñoz-Fuentes, V., Green, A.J., Wilson, R.E., and McCracken, K.G. (2018). Migration-selection balance drives genetic differentiation in genes associated with high-altitude function in the speckled teal (*Anas flavirostris*) in the Andes. Genome Biol. Evol. *10*, 14–32. https://doi.org/10.1093/gbe/evx253.
96. Palomera-Sanchez, Z., and Zurita, M. (2011). Open, repair and close again: chromatin dynamics and the response to UV-induced DNA damage. DNA Repair *10*, 119–125. https://doi.org/10.1016/j.dnarep.2010.10.010.
97. Yu, L., Wang, G.D., Ruan, J., Chen, Y.B., Yang, C.P., Cao, X., Wu, H., Liu, Y.H., Du, Z.L., Wang, X.P., et al. (2016). Genomic analysis of snub-nosed monkeys (*Rhinopithecus*) identifies genes and processes related to high-altitude adaptation. Nat. Genet. *48*, 947–952. https://doi.org/10.1038/ng.3615.
98. Guo, W., Xin, M., Wang, Z., Yao, Y., Hu, Z., Song, W., Yu, K., Chen, Y., Wang, X., Guan, P., et al. (2020). Origin and adaptation to high altitude of Tibetan semi-wild wheat. Nat. Commun. *11*, 5085. https://doi.org/10.1038/s41467-020-18738-5.
99. Xiong, Y., and Lei, F. (2019). High-altitude adaptation in birds: From phenomics to genomics. Sci. Sin. -Vitae. *49*, 361–370. https://doi.org/10.1360/N052018-00203.
100. Yang, W., Qi, Y., and Fu, J. (2014). Exploring the genetic basis of adaptation to high elevations in reptiles: a comparative transcriptome analysis of two toad-headed agamas (genus *Phrynocephalus*). PLoS One *9*, e112218. https://doi.org/10.1371/journal.pone.0112218.
101. Zhang, Q.L., Zhang, L., Yang, X.Z., Wang, X.T., Li, X.P., Wang, J., Chen, J.Y., and Yuan, M.L. (2017). Comparative transcriptomic analysis of Tibetan *Gynaephora* to explore the genetic basis of insect adaptation to divergent altitude environments. Sci. Rep. *7*, 16972. https://doi.org/10.1038/s41598-017-17051-4.
102. Zhang, Q.L., Li, H.W., Dong, Z.X., Yang, X.J., Lin, L.B., Chen, J.Y., and Yuan, M.L. (2020). Comparative transcriptomic analysis of fireflies (Coleoptera: Lampyridae) to explore the molecular adaptations to fresh water. Mol. Ecol. *29*, 2676–2691. https://doi.org/10.1111/mec.15504.
103. Stork, N.E. (2018). How many species of insects and other terrestrial arthropods are there on Earth? Annu. Rev. Entomol. *63*, 31–45. https://doi.org/10.1146/annurev-ento-020117-043348.
104. Harrison, M.C., Jongepier, E., Robertson, H.M., Arning, N., Bitard-Feildel, T., Chao, H., Childers, C.P., Dinh, H., Doddapaneni, H., Dugan, S., et al. (2018). Hemimetabolous genomes reveal molecular basis of termite eusociality. Nat. Ecol. Evol. *2*, 557–566. https://doi.org/10.1038/s41559-017-0459-1.
105. Poulsen, M., Hu, H., Li, C., Chen, Z., Xu, L., Otani, S., Nygaard, S., Nobre, T., Klaubauf, S., Schindler, P.M., et al. (2014). Complementary symbiont contributions to plant decomposition in a fungus-farming termite. Proc. Natl. Acad. Sci. USA *111*, 14500–14505. https://doi.org/10.1073/pnas.1319718111.
106. Terrapon, N., Li, C., Robertson, H.M., Ji, L., Meng, X., Booth, W., Chen, Z., Childers, C.P., Glastad, K.M., Gokhale, K., et al. (2014). Molecular traces of alternative social organization in a termite genome. Nat. Commun. *5*, 3636. https://doi.org/10.1038/ncomms4636.
107. Watanabe, H., Noda, H., Tokuda, G., and Lo, N. (1998). A cellulase gene of termite origin. Nature *394*, 330–331. https://doi.org/10.1038/28527.
108. Pedezzi, R., Fonseca, F.P.P., Santos Júnior, C.D., Kishi, L.T., Terra, W.R., and Henrique-Silva, F. (2014). A novel β-fructofuranosidase in Coleoptera: Characterization of a β-fructofuranosidase from the sugarcane weevil, *Sphenophorus levis*. Insect Biochem. Mol. Biol. *55*, 31–38. https://doi.org/10.1016/j.ibmb.2014.10.005.
109. Nagare, M., Ayachit, M., Agnihotri, A., Schwab, W., and Joshi, R. (2021). Glycosyltransferases: the multifaceted enzymatic regulator in insects. Insect Mol. Biol. *30*, 123–137. https://doi.org/10.1111/imb.12686.
110. Daimon, T., Hirayama, C., Kanai, M., Ruike, Y., Meng, Y., Kosegawa, E., Nakamura, M., Tsujimoto, G., Katsuma, S., and Shimada, T. (2010). The silkworm Green b locus encodes a quercetin 5-O-glucosyltransferase that produces green cocoons with UV-shielding properties. Proc. Natl. Acad. Sci. USA *107*, 11471–11476. https://doi.org/10.1073/pnas.1000479107.
111. de Vries, R.P., and Visser, J. (2001). *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides. Microbiol. Mol. Biol. Rev. *65*, 497–522. https://doi.org/10.1128/mmbr.65.4.497-522.2001.
112. Biedermann, P.H.W., and Vega, F.E. (2020). Ecology and Evolution of Insect-Fungus Mutualisms. Annu. Rev. Entomol. *65*, 431–455. https://doi.org/10.1146/annurev-ento-011019-024910.
113. Jagadeeswaran, G., Veale, L., and Mort, A.J. (2021). Do lytic polysaccharide monooxygenases aid in plant pathogenesis and herbivory? Trends Plant Sci. *26*, 142–155. https://doi.org/10.1016/j.tplants.2020.09.013.
114. Sabbadin, F., Hemsworth, G.R., Ciano, L., Henrissat, B., Dupree, P., Tryfona, T., Marques, R.D.S., Sweeney, S.T., Besser, K., Elias, L., et al. (2018). An ancient family of lytic polysaccharide monooxygenases with roles in arthropod development and biomass digestion. Nat. Commun. *9*, 756. https://doi.org/10.1038/s41467-018-03142-x.
115. Keeling, C.I., Yuen, M.M.S., Liao, N.Y., Docking, T.R., Chan, S.K., Taylor, G.A., Palmquist, D.L., Jackman, S.D., Nguyen, A., Li, M., et al. (2013). Draft genome of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, a major forest pest. Genome Biol. *14*, R27. https://doi.org/10.1186/gb-2013-14-3-r27.
116. Zhao, T., Kandasamy, D., Krokene, P., Chen, J., Gershenzon, J., and Hammerbacher, A. (2019). Fungal associates of the tree-killing bark beetle, *Ips typographus*, vary in virulence, ability to degrade conifer phenolics and influence bark beetle tunneling behavior. Fungal Ecol. *38*, 71–79.
117. Bolger, A.M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for

Illumina sequence data. Bioinformatics *30*, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

118. Marçais, G., and Kingsford, C. (2011). A fast, lock-free approach for efficient parallel counting of occurrences of k-mers. Bioinformatics *27*, 764–770. https://doi.org/10.1093/bioinformatics/btr011.
119. Ranallo-Benavidez, T.R., Jaron, K.S., and Schatz, M.C. (2020). GenomeScope 2.0 and Smudgeplot for reference-free profiling of polyploid genomes. Nat. Commun. *11*, 1432–1510. https://doi.org/10.1038/s41467-020-14998-3.
120. Koren, S., Walenz, B.P., Berlin, K., Miller, J.R., Bergman, N.H., and Phillippy, A.M. (2017). Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Res. *27*, 722–736. https://doi.org/10.1101/gr.215087.116.
121. Guan, D., McCarthy, S.A., Wood, J., Howe, K., Wang, Y., and Durbin, R. (2020). Identifying and removing haplotypic duplication in primary genome assemblies. Bioinformatics *36*, 2896–2898. https://doi.org/10.1093/bioinformatics/btaa025.
122. Walker, B.J., Abeel, T., Shea, T., Priest, M., Abouelliel, A., Sakthikumar, S., Cuomo, C.A., Zeng, Q., Wortman, J., Young, S.K., and Earl, A.M. (2014). Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One *9*, e112963. https://doi.org/10.1371/journal.pone.0112963.
123. Durand, N.C., Shamim, M.S., Machol, I., Rao, S.S.P., Huntley, M.H., Lander, E.S., and Aiden, E.L. (2016). Juicer provides a one-click system for analyzing loop-resolution Hi-C experiments. Cell Syst. *3*, 95–98. https://doi.org/10.1016/j.cels.2016.07.002.
124. Dudchenko, O., Batra, S.S., Omer, A.D., Nyquist, S.K., Hoeger, M., Durand, N.C., Shamim, M.S., Machol, I., Lander, E.S., Aiden, A.P., and Aiden, E.L. (2017). *De novo* assembly of the *Aedes aegypti* genome using Hi-C yields chromosome-length scaffolds. Science *356*, 92–95. https://doi.org/10.1126/science.aal3327.
125. Durand, N.C., Robinson, J.T., Shamim, M.S., Machol, I., Mesirov, J.P., Lander, E.S., and Aiden, E.L. (2016). Juicebox provides a visualization system for Hi-C contact maps with unlimited zoom. Cell Syst. *3*, 99–101. https://doi.org/10.1016/j.cels.2015.07.012.
126. Simão, F.A., Waterhouse, R.M., Ioannidis, P., Kriventseva, E.V., and Zdobnov, E.M. (2015). BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics *31*, 3210–3212. https://doi.org/10.1093/bioinformatics/btv351.
127. Bedell, J.A., Korf, I., and Gish, W. (2000). *MaskerAid*: A performance enhancement to RepeatMasker. Bioinformatics *16*, 1040–1041. https://doi.org/10.1093/bioinformatics/16.11.1040.
128. Tarailo-Graovac, M., and Chen, N. (2009). Using RepeatMasker to identify repetitive elements in genomic sequences. Curr. Protoc. Bioinformatics *25*, 4. https://doi.org/10.1002/0471250953.bi0410s25.
129. Slater, G.S.C., and Birney, E. (2005). Automated generation of heuristics for biological sequence comparison. BMC Bioinf. *6*, 31. https://doi.org/10.1186/1471-2105-6-31.
130. Ter-Hovhannisyan, V., Lomsadze, A., Chernoff, Y.O., and Borodovsky, M. (2008). Gene prediction in novel fungal genomes using an *ab initio* algorithm with unsupervised training. Genome Res. *18*, 1979–1990. https://doi.org/10.1101/gr.081612.108.
131. Stanke, M., Steinkamp, R., Waack, S., and Morgenstern, B. (2004). AUGUSTUS: a web server for gene finding in eukaryotes. Nucleic Acids Res. *32*, W309–W312. https://doi.org/10.1093/nar/gkh379.
132. Haas, B.J., Salzberg, S.L., Zhu, W., Pertea, M., Allen, J.E., Orvis, J., White, O., Buell, C.R., and Wortman, J.R. (2008). Automated eukaryotic gene structure annotation using EVidenceModeler and the program to assemble spliced alignments. Genome Biol. *9*, R7–R22. https://doi.org/10.1186/gb-2008-9-1-r7.
133. Haas, B.J., Delcher, A.L., Mount, S.M., Wortman, J.R., Smith, R.K., Hannick, L.I., Maiti, R., Ronning, C.M., Rusch, D.B., Town, C.D., et al. (2003). Improving the *Arabidopsis* genome annotation using maximal transcript alignment assemblies. Nucleic Acids Res. *31*, 5654–5666. https://doi.org/10.1093/nar/gkg770.
134. Nawrocki, E.P. (2014). Annotating functional RNAs in genomes using Infernal. Methods Mol. Biol. *1097*, 163–197. https://doi.org/10.1007/978-1-62703-709-9_9.
135. Lowe, T.M., and Eddy, S.R. (1997). tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. *25*, 955–964. https://doi.org/10.1093/nar/25.5.955.
136. Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. *25*, 3389–3402. https://doi.org/10.1093/nar/25.17.3389.
137. Meng, X., and Ji, Y. (2013). Modern computational techniques for the HMMER sequence analysis. ISRN Bioinform. *2013*, 252183. https://doi.org/10.1155/2013/252183.
138. Aramaki, T., Blanc-Mathieu, R., Endo, H., Ohkubo, K., Kanehisa, M., Goto, S., and Ogata, H. (2020). KofamKOALA: KEGG ortholog assignment based on profile HMM and adaptive score threshold. Bioinformatics *36*, 2251–2252. https://doi.org/10.1093/bioinformatics/btz859.
139. Emms, D.M., and Kelly, S. (2019). OrthoFinder: phylogenetic orthology inference for comparative genomics. Genome Biol. *20*, 238. https://doi.org/10.1186/s13059-019-1832-y.
140. Katoh, K., Misawa, K., Kuma, K.i., and Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. *30*, 3059–3066. https://doi.org/10.1093/nar/gkf436.
141. Talavera, G., and Castresana, J. (2007). Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol. *56*, 564–577. https://doi.org/10.1080/10635150701472164.
142. Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics *30*, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033.
143. Yang, Z. (2007). PAML 4: phylogenetic analysis by maximum likelihood. Mol. Biol. Evol. *24*, 1586–1591. https://doi.org/10.1093/molbev/msm088.
144. De Bie, T., Cristianini, N., Demuth, J.P., and Hahn, M.W. (2006). CAFE: a computational tool for the study of gene family evolution. Bioinformatics *22*, 1269–1271. https://doi.org/10.1093/bioinformatics/btl097.
145. Alexa, A., Rahnenführer, J., and Lengauer, T. (2006). Improved scoring of functional groups from gene expression data by decorrelating GO graph structure. Bioinformatics *22*, 1600–1607. https://doi.org/10.1093/bioinformatics/btl140.
146. Xie, C., Mao, X., Huang, J., Ding, Y., Wu, J., Dong, S., Kong, L., Gao, G., Li, C.Y., and Wei, L. (2011). KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. Nucleic Acids Res. *39*, W316–W322. https://doi.org/10.1093/nar/gkr483.
147. Edgar, R.C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. *32*, 1792–1797. https://doi.org/10.1093/nar/gkh340.
148. Suyama, M., Torrents, D., and Bork, P. (2006). PAL2NAL: robust conversion of protein sequence alignments into the corresponding codon alignments. Nucleic Acids Res. *34*, W609–W612. https://doi.org/10.1093/nar/gkl315.
149. Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., et al. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. *29*, 644–652. https://doi.org/10.1038/nbt.1883.
150. Kim, D., Paggi, J.M., Park, C., Bennett, C., and Salzberg, S.L. (2019). Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nat. Biotechnol. *37*, 907–915. https://doi.org/10.1038/s41587-019-0201-4.
151. Liao, Y., Smyth, G.K., and Shi, W. (2014). featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics *30*, 923–930.
152. Calderón-Cortés, N., Quesada, M., Cano-Camacho, H., and Zavala-Páramo, G. (2010). A simple and rapid method for DNA isolation from xylophagous insects. Int. J. Mol. Sci. *11*, 5056–5064. https://doi.org/10.3390/ijms11125056.
153. Belaghzal, H., Dekker, J., and Gibcus, J.H. (2017). Hi-C 2.0: An optimized Hi-C procedure for high-resolution genome-wide mapping of chromosome conformation. Methods *123*, 56–65. https://doi.org/10.1016/j.ymeth.2017.04.004.
154. Bao, W., Kojima, K.K., and Kohany, O. (2015). Repbase Update, a database of repetitive elements in eukaryotic genomes. Mob. DNA *6*, 11–16. https://doi.org/10.1186/s13100-015-0041-9.
155. Kalvari, I., Nawrocki, E.P., Ontiveros-Palacios, N., Argasinska, J., Lamkiewicz, K., Marz, M., Griffiths-Jones, S., Toffano-Nioche, C., Gautheret, D., Weinberg, Z., et al. (2021). Rfam 14: expanded coverage of metagenomic, viral and microRNA

families. Nucleic Acids Res. *49*, D192–D200. https://doi.org/10.1093/nar/gkaa1047.

156. Huerta-Cepas, J., Szklarczyk, D., Heller, D., Hernández-Plaza, A., Forslund, S.K., Cook, H., Mende, D.R., Letunic, I., Rattei, T., Jensen, L.J., et al. (2019). eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. Nucleic Acids Res. *47*, D309–D314. https://doi.org/10.1093/nar/gky1085.

157. Zhang, S., Shen, S., Peng, J., Zhou, X., Kong, X., Ren, P., Liu, F., Han, L., Zhan, S., Huang, Y., et al. (2020). Chromosome-level genome assembly of an important pine defoliator, *Dendrolimus punctatus* (Lepidoptera; Lasiocampidae). Mol. Ecol. Resour. *20*, 1023–1037. https://doi.org/10.1111/1755-0998.13169.

158. Zhang, H., Yohe, T., Huang, L., Entwistle, S., Wu, P., Yang, Z., Busk, P.K., Xu, Y., and Yin, Y. (2018). dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. Nucleic Acids Res. *46*, W95–W101. https://doi.org/10.1093/nar/gky418.

159. Darriba, D., Taboada, G.L., Doallo, R., and Posada, D. (2011). ProtTest 3: fast selection of best-fit models of protein evolution. Bioinformatics *27*, 1164–1165. https://doi.org/10.1093/bioinformatics/btr088.

160. Parkhomchuk, D., Borodina, T., Amstislavskiy, V., Banaru, M., Hallen, L., Krobitsch, S., Lehrach, H., and Soldatov, A. (2009). Transcriptome analysis by strand-specific sequencing of complementary DNA. Nucleic Acids Res. *37*, e123. https://doi.org/10.1093/nar/gkp596.

161. Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics *26*, 139–140.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Hi-C sequencing data	This study	NCBI: SRR14269884
PacBio sequencing data	This study	NCBI: SRR14269885
Illumina sequencing data	This study	NCBI: SRR14269886
Transcriptome raw data	This study	NCBI: SRR18907819-SRR18907827, SRR23085891-SRR23085893
Genome assembly data	This study	NCBI: JAGUCG020000000
Aethina tumida reference genome	NCBI	GCA_001937115.1
Apis mellifera reference genome	NCBI	GCA_003254395.2
Agrilus planipennis reference genome	NCBI	GCA_000699045.2
Anoplophora glabripennis reference genome	NCBI	GCA_000390285.2
Dendroctonus ponderosae reference genome	NCBI	GCA_000355655.1
Dendrolimus punctatus reference genome	NCBI	GCA_012273795.1
Diabrotica virgifera reference genome	NCBI	GCA_003013835.2
Hypothenemus hampei reference genome	NCBI	GCA_001012855.1
Ips typographus reference genome	NCBI	GCA_016097725.1
Nicrophorus vespilloides reference genome	NCBI	GCA_001412225.1
Onthophagus taurus reference genome	NCBI	GCA_000648695.2
Sitophilus oryzae reference genome	NCBI	GCA_002938485.2
Thaumetopoea pityocampa reference genome	NCBI	GCA_017165845.1
Tribolium castaneum reference genome	NCBI	GCA_000002335.3
Software and algorithms		
Trimmomatic v0.39	Bolger et al.[117]	https://github.com/usadellab/Trimmomatic/releases
Jellyfish v2.3.0	Marçais and Kingsford[118]	https://github.com/gmarcais/Jellyfish
GenomeScope 2.0	Ranallo-Benavidez et al.[119]	http://qb.cshl.edu/genomescope/genomescope2.0/
Canu v2.1	Koren et al.[120]	https://github.com/marbl/canu
Purge_dups v1.0.1	Guan et al.[121]	https://github.com/dfguan/purge_dups/releases
GCpp v1.9.0		https://github.com/PacificBiosciences/gcpp
Pilon v1.23	Walker et al.[122]	https://github.com/broadinstitute/pilon
Juicer v1.6	Durand et al.[123]	https://github.com/aidenlab/juicer
3D-DNA pipeline v180922	Dudchenko et al.[124]	https://github.com/aidenlab/3d-dna
JBAT v.1.8.8	Durand et al.[125]	https://github.com/aidenlab/Juicebox
BUSCO v3.1.0	Simão et al.[126]	https://busco.ezlab.org/
RepeatModeler v2.0.1	Bedell et al.[127]	https://www.repeatmasker.org/RepeatModeler/
RepeatMask v4.1.1	Tarailo-Graovac and Chen[128]	https://www.repeatmasker.org/
Exonerate v2.2.0	Slater and Birney[129]	https://github.com/nathanweeks/exonerate
GeneMark v4.61	Ter-Hovhannisyan et al.[130]	https://topaz.gatech.edu/GeneMark/
Augustus v3.3.3	Stanke et al.[131]	https://github.com/Gaius-Augustus/Augustus
EVidenceModeler v1.1.1	Haas et al.[132]	https://github.com/EVidenceModeler/EVidenceModeler
PASA v2.4.1	Haas et al.[133]	https://github.com/PASApipeline/PASApipeline/releases

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Infernal v1.1.3	Nawrocki[134]	https://eddylab.org/infernal/
tRNAscan-SE v2.0.7	Lowe and Eddy[135]	https://github.com/UCSC-LoweLab/tRNAscan-SE
BLASTP v2.10.1+	Altschul et al.[136]	https://blast.ncbi.nlm.nih.gov/doc/blast-help/downloadblastdata.html#downloadblastdata
Interproscan v5.33		https://www.ebi.ac.uk/interpro/about/interproscan/
HMMER v3.3.1	Meng and Ji[137]	http://hmmer.org/
KofamScan v1.3.0	Aramaki et al.[138]	https://anaconda.org/bioconda/kofamscan
OrthoFinder v 2.4.0	Emms and Kelly[139]	https://github.com/davidemms/OrthoFinder
MAFFT v7.471	Katoh et al.[140]	https://github.com/GSLBiotech/mafft
GBLOCKS v0.91b	Talavera and Castresana[141]	https://home.cc.umanitoba.ca/~psgendb/doc/Castresana/Gblocks_documentation.html
RAxML v8.2.12	Stamatakis[142]	https://cme.h-its.org/exelixis/web/software/raxml/
PAML v4.9j	Yang[143]	https://abacus.gene.ucl.ac.uk/software/paml.html
Café v4.2.1	De et al.[144]	https://hahnlab.github.io/CAFE/src_docs/html/index.html
topGO v2.40.0	Alexa et al.[145]	https://bioconductor.org/packages/release/bioc/html/topGO.html
KOBAS v3.0	Xie et al.[146]	http://kobas.cbi.pku.edu.cn/
MUSCLE	Edgar[147]	https://www.ebi.ac.uk/Tools/msa/muscle/
PAL2NAL	Suyama et al.[148]	http://www.bork.embl.de/pal2nal/
FastQC v0.11.9		https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
Trinity v2.11.0	Grabherr et al.[149]	https://github.com/trinityrnaseq/trinityrnaseq/
HISAT2 v2.2.1	Kim et al.[150]	https://github.com/DaehwanKimLab/hisat2/releases
FeatureCounts v2.0.3	Liao et al.[151]	https://subread.sourceforge.net/featureCounts.html

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Quan Lu (luquan@caf.ac.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The accession numbers for the genomic sequencing data, Transcriptome data, and genome assembly reported in this paper were listed in the key resources table.

The published article and supplemental information include all data generated and analyzed during this study. This paper does not report original code.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Ips nitidus adults were collected from *Picea crassifolia* in Maixiu National Forest Park, Zeku County, Huangnan City, Qinghai Province, China (Average annual temperature was 2.8°C; The average temperature in the hottest month (July) and the coldest month (January) were 12.3°C and minus 10.1°C, respectively). The sampling site was 3,016 m above sea level (N: 35°16′28″ E: 101°54′46″). Thirty adults from the same parent in the same gallery were selected to minimize heterozygosity. To reduce microbial contamination, adults were starved for 48 h, placed in sterile Eppendorf tubes pre-filled with 75% alcohol, and placed on a scroll oscillator for 1 min for complete disinfection. Five times, the beetles were rinsed with sterile water to remove any residual microorganisms. The residual water on the surface of the beetle body was removed by

gentle drying using sterile filter paper, and the heads of the 30 beetles were flash-frozen in liquid nitrogen for subsequent DNA extraction and sequencing. An additional 30 adult heads were obtained for Hi-C sequencing using the abovementioned method.

METHOD DETAILS

Genome sequencing

Genomic DNA from the pooled adult beetle heads was extracted following the CTAB-PVP method.[152] The integrity and quantity of DNA were determined using a 0.8% agarose gel and a Qubit fluorimeter (Invitrogen, Carlsbad, CA, USA). Prior to sequencing, PCR was performed using universal primers that amplified 16S rRNA and ITS sequences, respectively. No amplified DNA bands (PCR product) were observed, thus confirming no microbial contamination. High-quality DNA was split into equal parts for Illumina and Pacific Biosciences (PacBio) sequencing at Berry Genomics Company, China. A 400 bp insert length paired-end library was constructed for Illumina sequencing using the NEBNext® Ultra™ II DNA Library Prep Kit (New England Biolabs, Ipswich, MA, USA). The library was then sequenced on an Illumina HiSeq X Ten System to obtain 150 bp paired-end reads. For PacBio long-read sequencing, a 40-kb SMRTbell library was constructed following the manufacturer's protocol (Pacific Biosciences, Menlo Park, CA, USA) and then sequenced on the PacBio Sequel Systems platform.

We employed the Hi-C technique to improve scaffolding to generate a chromosome-level assembly. A Hi-C library was constructed with the DpnII restriction enzyme following *in situ* ligation protocols.[153] Briefly, the pieces of beetle heads were cross-linked with 2% formaldehyde for 10 minutes and then incubated with 2.5 M glycine solution for 5 minutes to stop cross-linking. The nuclei isolated from the fixed samples were digested with DpnII enzyme, the blunt-end was filled and marked with biotin-14-dATP (Thermo Fisher Scientific, Waltham, MA, USA) and then ligated. The ligated DNA was purified and sheared into 300-600 bp fragments. The segments were blunt-end repaired and A-tailed, followed by purification through biotin-streptavidin-mediated pull-down. After adaptor ligation and quality control, the Hi-C library was sequenced on an Illumina HiSeq X Ten System with the PE150 strategy.

Genome assembly and evaluation

Raw reads from Illumina sequencing were filtered to remove low-quality reads using Trimmomatic v0.39[117] with the parameter "AVGQUAL:30". The genome size of *I. nitidus* was estimated by k-mer analysis (k-mer size 21) based on clean reads from next-generation sequencing (NGS) with Jellyfish v2.3.0[118] using GenomeScope 2.0.[119] Initial genome assembly used PacBio long reads with Canu v2.1[120] and the parameters "-dg 3 -db 3 -dr 1 -ca 500 -cp50". Purge_dups v1.0.1[121] was employed with default parameters to resolve haplotype-fused paths in the initial assembly. After that, the resolved assembly was further refined to improve the accuracy. First, long reads were used to polish the resolved assembly with GCpp v1.9.0 (https://github.com/PacificBiosciences/gcpp), and then short reads were utilized with Pilon v1.23.[122]

To obtain a chromosome-level assembly of the *I. nitidus* genome, we performed Hi-C scaffolding with Juicer v1.6[123] and the 3D *de novo* assembly (3D-DNA) pipeline v180922.[124] Briefly, the polished PacBio initial assembly was digested *in silico* by DpnII enzyme using the python script "generate_site_positions.py" within Juicer. Raw reads from the Hi-C library were filtered using Trimmomatic v0.39 as NGS reads. The clean reads were aligned to the draft genome and processed to generate Hi-C contacts using Juicer with parameters "-s DpnII". The generated contact files (merged_nodups.txt) were used to assemble the chromosome-level genome with a 3D-DNA pipeline with the default parameters. The mega-scaffold output was then imported to Juicebox Assembly Tools (JBAT) v. 1.8.8[125] for manual review and refinement. The completeness of the genome assembly was determined using BUSCO v3.1.0[126] against 1367 benchmarking universal single-copy orthologous genes in insecta_odb10.

Genome annotation

Repeat sequences were identified using *de novo* prediction. RepeatModeler v2.0.1[127] was initially used to construct a *de novo* repeat library with default parameters. Then, RepeatMask v4.1.1[128] was applied with default parameters to identify homology repeats based on the combination of the *de novo* library and Repbase library (20181026).[154]

We employed homology-based, *de novo*, and RNA-assisted methods for protein-coding gene structure prediction. Briefly, proteins from *Dendroctonus ponderosae* (Keeling et al., 2013) were aligned to the *I. nitidus* genome using Exonerate v2.2.0[129] with default parameters to identify intron and exon boundaries. For *de novo* gene prediction, GeneMark v4.61[130] and Augustus v3.3.3[131] were used. GeneMark was performed using a self-training algorithm. Augustus was used with training sets that combined the gene structures from Exonerate and GeneMark. The consensus gene set was generated using EVidenceModeler v1.1.1.[132] Finally, PASApipeline v2.4.1[133] was used to upgrade the gene models with the *de novo* transcriptome. For ncRNA prediction, we scanned the Rfam database (release 14.0)[155] with Infernal v1.1.3[134] and tRNAscan-SE v2.0.7[135] was used for tRNA prediction.

We assigned putative functions to newly annotated genes by aligning the genes from *I. nitidus* using BLASTP v2.10.1+[136] with an E-value of 1e-5 to eggNOG 5.0,[156] NCBI non-redundant amino acid sequences (NR) (assessed 202006), Swiss-Prot (2020_05), and UniRef50 (2020_06). Interproscan v5.33 was used to scan InterPro 72.0. HMMER v3.3.1[137] was used to align against CAZy v9 and Pfam 33.1. Furthermore, the genes were aligned against the Kyoto Encyclopaedia of Genes and Genomes (KEGG) database using KofamScan v1.3.0.[138] Gene Ontology (GO) annotation was extracted from Gene Ontology Annotation (GOA, 2020_10) based on Swiss-Prot.

Phylogenetic inference and comparative genomic analysis

Orthologous and paralogous groups were identified using OrthoFinder v 2.4.0[139] with default parameters from *I. nitidus* and 14 other insects, including 11 species of Coleoptera (*Aethina tumida*, *Agrilus planipennis*, *Anoplophora glabripennis*, *D. ponderosae*, *Diabrotica virgifera*, *Hypothenemus hampei*, *I. typographus*, *Nicrophorus vespilloides*, *Onthophagus taurus*, *Sitophilus oryzae*, and *Tribolium castaneum*), two species of Lepidoptera (*Dendrolimus punctatus* and *Thaumetopoea pityocampa*), which feed on pinaceous plants, and one species of Hymenoptera (*Apis mellifera*). Among them, *Ap. mellifera* served as an outgroup. The longest protein was nominated for the genes with redundant alternative splicing events. The protein sequences of each single-copy group were extracted, and multiple sequence alignment was performed using MAFFT v7.471[140] with default parameters and then concatenated to generate a supermatrix. GBLOCKS v0.91b[141] was used to extract the conserved blocks with the default settings. A phylogenetic tree was constructed using RAxML v8.2.12[142] under the GAMMALG model with 1,000 bootstrap replicates. The estimated divergence time based on the topology structure of the phylogenetic tree was performed using MCMCTREE within PAML v4.9j, with five secondary calibration points selected.[143] The divergence time between Coleoptera and Hymenoptera was constrained to 317.80–372.43 million years ago (mya); between Coleoptera and Lepidoptera at 301.86–353.05 mya; between *An. glabripennis* and *Di. virgifera* at 122.50–124.00 mya; between *An. glabripennis* and *S. oryzae* at 157.30–166.10 mya; between *De. punctatus* and *Th. pityocampa* at 99.72–121.64 mya.[14,48,157] Contraction and expansion families were identified using Café v4.2.1.[144] In CAFE, the separate birth (λ) and death (μ) rates were estimated using the lambda/mu command with -s and –t 10. All *p*-values were calculated for each gene family, and a threshold of p-value < 0.05 was used as the criterion to identify the contraction and expansion of gene families. Subsequently, functional enrichment analysis was performed for the above gene families. GO functional enrichment analysis was performed using the weight method with Fisher's exact test implemented in the topGO v2.40.0.[145] KEGG enrichment analysis was performed using KOBAS v3.0.[146]

The orthogroups identified by OrthoFinder were further annotated for plant cell wall degrading enzymes (PCWDEs) and detoxification-related gene families comparison. The protein sequence with the longest length within each orthogroup was extracted as a representative sequence. PCWDEs were predicted using dbCAN2 with HMMER v3.3.1[158] and manually identified based on their CAZy classification (http://www.cazy.org). The detoxification-related gene families were predicted using HMMER by searching against the Pfam 33.1 (18259 different families) with parameter: -E 1e-5 –domE 1e-5.

We selected the protein sequences encoded by single-copy orthologous genes in four Curculionidae species (*I. nitidus*, *I. typographus*, *D. ponderosae* and *S. oryzae*) to align by MUSCLE[147] with the default parameters, respectively. The protein multiple sequence alignment results were used to generate the codon multiple sequence alignment by PAL2NAL.[148] The codeml program with the branch-site model was conducted in the PAML[159] to determine which genes were positively selected in *I. nitidus*. Chi-squared test was conducted to determine the statistical significance, and all positively selected genes were below p-value < 0.05 and FDR < 0.05 cut-off.

Choice aassay with fungus-free or fungus-colonized semi-natural spruce bark substrates

Fresh inner bark from healthy *P. crassifolia* was ground into a fine powder and used to prepare semi-natural spruce bark substrates. The substrates were prepared according to the description by Kandasamy et al.[83]: 7% fine powder of inner bark mixed with 4% agar (Mix 70 g bark powder with 40 g agar and add deionized water to 1000 ml) and dispensed in 9 cm Petri dishes after autoclaving.

For fungus-colonized treatments, a round mycelium plug (5 mm diam) from an actively growing *O. bicolor* (the dominant associate of *I. nitidus*) colony was placed in the center of a 9 cm Petri dish containing sterile semi-natural spruce bark substrate. For fungus-free treatments, the plug in the above procedure was sterile. All cultures were incubated in darkness at 25°C for seven days to prepare the choice arenas, which contained two semicircles of substrate separated by a 5 mm wide strip of sterile 4% agar.

Callow adult beetles were starved for 24 h at 4°C and surface sterilized in 1.2% NaClO and 2% ethanol for 30 s, followed by three rinses in sterile water prior to bioassays.[83] Four vibrant beetles were placed in agar strips in each choice arena to start their choice. Nine choice arena replicates were set up and placed in a climate chamber at 20°C and in complete darkness. After 24 hours, the preferences of the beetles were recorded, and the data were statistically analyzed using SPSS v10.0.1 with a one-way analysis of variance (ANOVA).

Transcriptome sequencing and analysis

Four different treatments of adult beetles were used to compare transcriptomes: 1) feeding and tunnelling in fungus-colonized (mark: DGQM) and 2) fungus-free substrates (mark: DGQA) placed in a climate chamber at low altitudes (approximately 50 m above sea level), 3) feeding and tunnelling in fungus-colonized (mark: GGQM) and 4) fungus-free substrates (mark: GGQA) placed in a climate chamber at high altitudes (approximately 3000 m above sea level). Each treatment consisted of nine Petri dishes containing substrates, three of which were pooled as one biological replicate for a total of three biological replicates. The preparation of the beetles and substrates before inoculation is the same as described in the previous section. After inoculating six beetles, Petri dishes containing different substrates were placed in the climate chamber with 20°C and darkness. The high-altitude and low-altitude climate chambers maintained air circulation with the external environment to ensure that the oxygen concentration in the artificial climate chamber was consistent with that in the respective external environment. After 24 hours, the beetles were carefully and quickly removed from the substrates, and then surface sterilised in 75% ethanol for 30 s, followed by three rinses in sterile water. After soaking up the residual water on the surface of the beetle body with sterile filter paper, the beetles were snap-frozen in liquid nitrogen for subsequent RNA extraction and sequencing.

Total RNA was isolated from each sample using the Gene-JET plant RNA purification mini kit (Thermo Fisher Scientific, Waltham, MA, USA) and then quantified using a NanoDrop ND-2000 (Thermo Fisher Scientific, Waltham, MA, USA). RNA integrity was assessed using an Agilent

Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). Twelve RNA libraries with ~350 bp insert sizes were constructed using TruSeq RNA Sample Preparation Kit v2 (Illumina, San Diego, CA, USA) and the deoxyuridine triphosphate (dUTP) method.[160] All libraries were sequenced on an Illumina HiSeq 4000 System with the PE150 strategy. The quality of raw sequencing reads was assessed using FastQC v0.11.9 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) with default parameters and trimmed using Trimmomatic v0.39[117] with parameter 'AVGQUAL:30'. The output clean reads were used for two purposes: (a) *de novo* assembly for updating gene models and (b) identification of differentially expressed genes (DEGs). In short, Trinity v2.11.0[149] was used with default parameters for *de novo* assembly of transcripts to update gene models. To identify DEGs, the clean reads were aligned to the *I. nitidus* genome using HISAT2 v2.2.1[150] with default parameters. FeatureCounts v2.0.3 was used to calculate raw read counts of genes using gtf annotations with parameter -p –primary.[151] DEGs were identified using the R package edgeR v3.30.3.[161] For edgeR, the function of calcNorm-Factors and estimateDisp were used to calculate normalization factors and to estimate dispersion, respectively. DEGs were determined using a likelihood ratio test for a negative binomial generalized log-linear model with a threshold p-value < 0.05, FDR < 0.05, and $|\log2(\text{fold change})| \geq 1$.

QUANTIFICATION AND STATISTICAL ANALYSIS

Quantification and statistical analysis can be found in the method details.