

Soil-derived, gut-dominant generalist bacteria shape the fitness of folivorous larvae

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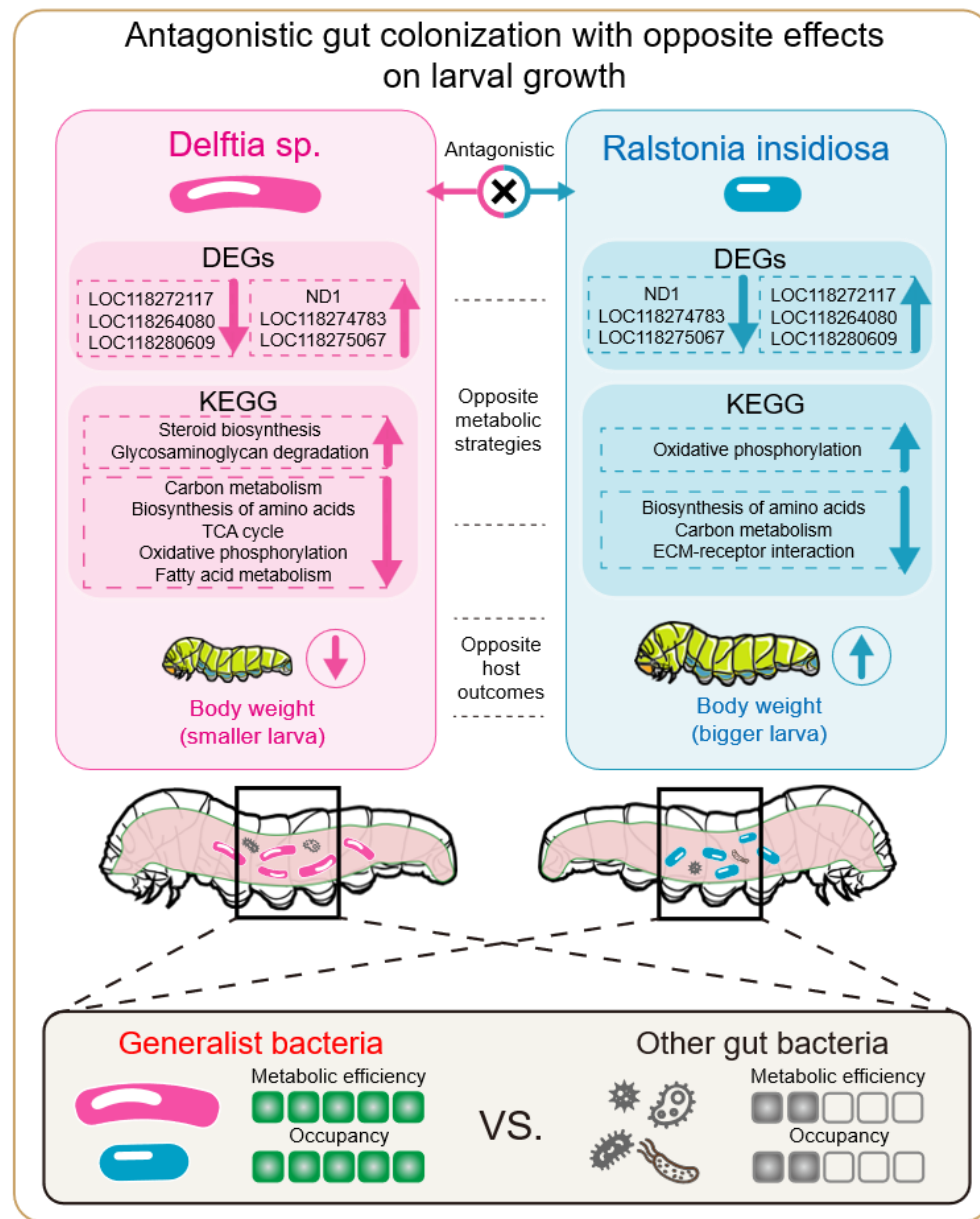
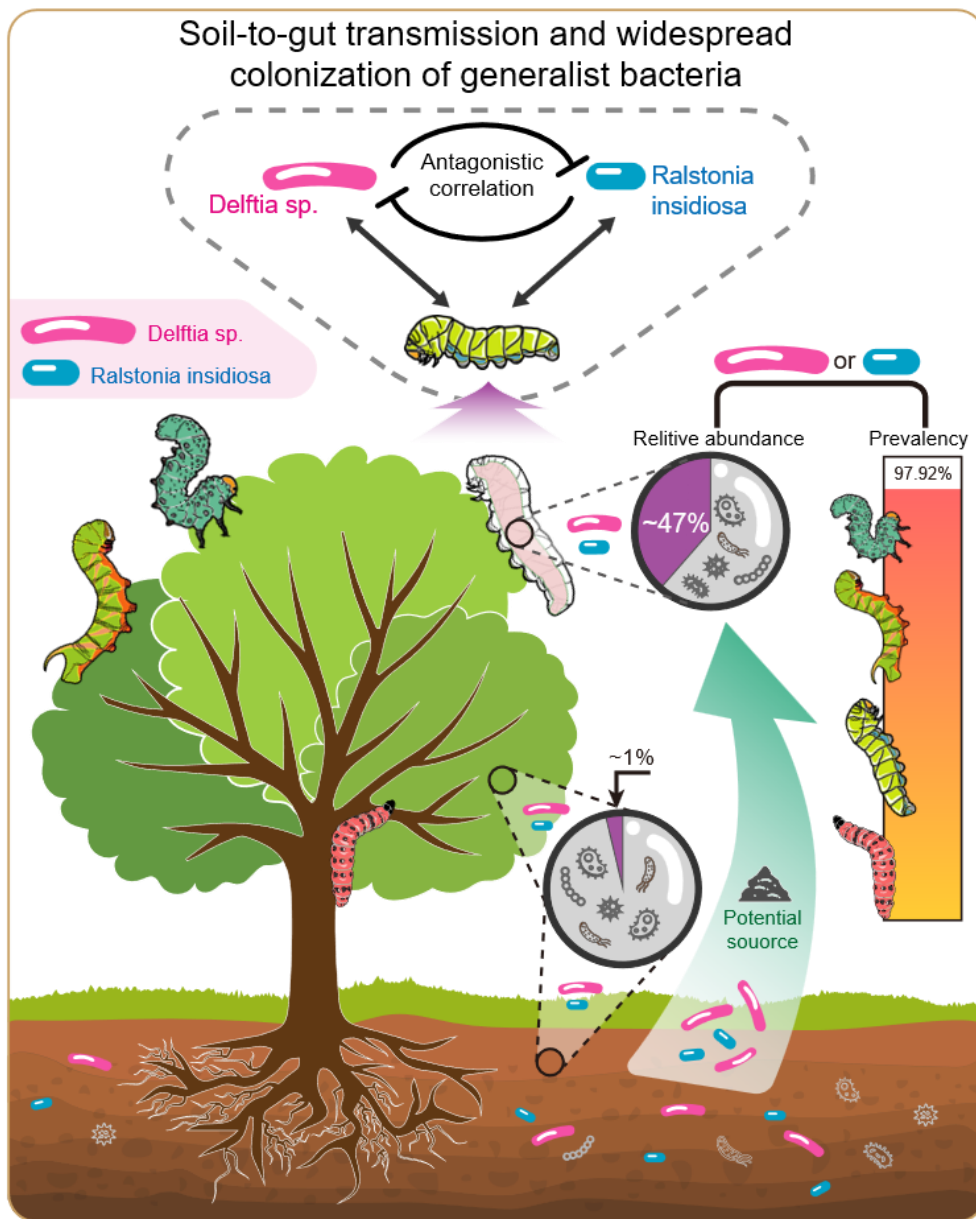


Highlights

- Two soil-derived “generalist”, *Ralstonia insidiosa* and *Delftia* sp., gut bacteria are highly prevalent in folivorous larvae and exhibit a strong mutually exclusive pattern in the larval gut;
- The two generalist bacteria exert contrasting physiological effects on host larval development—*Ralstonia insidiosa* promotes larval growth, whereas *Delftia* sp. inhibits larval growth.



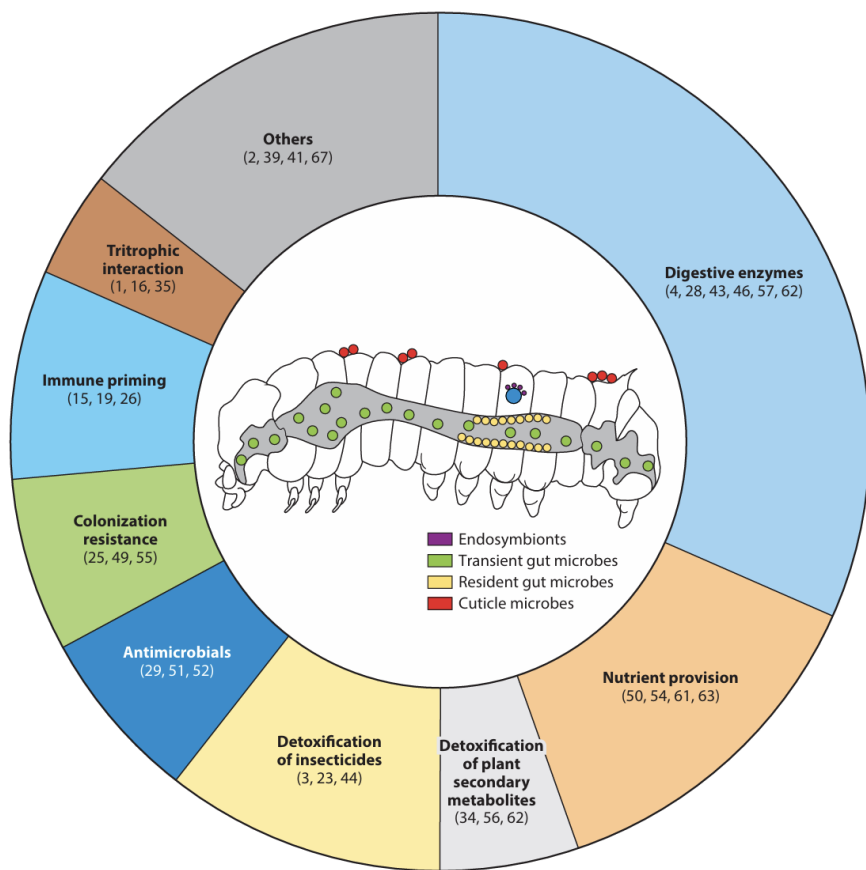
Graphical Abstract



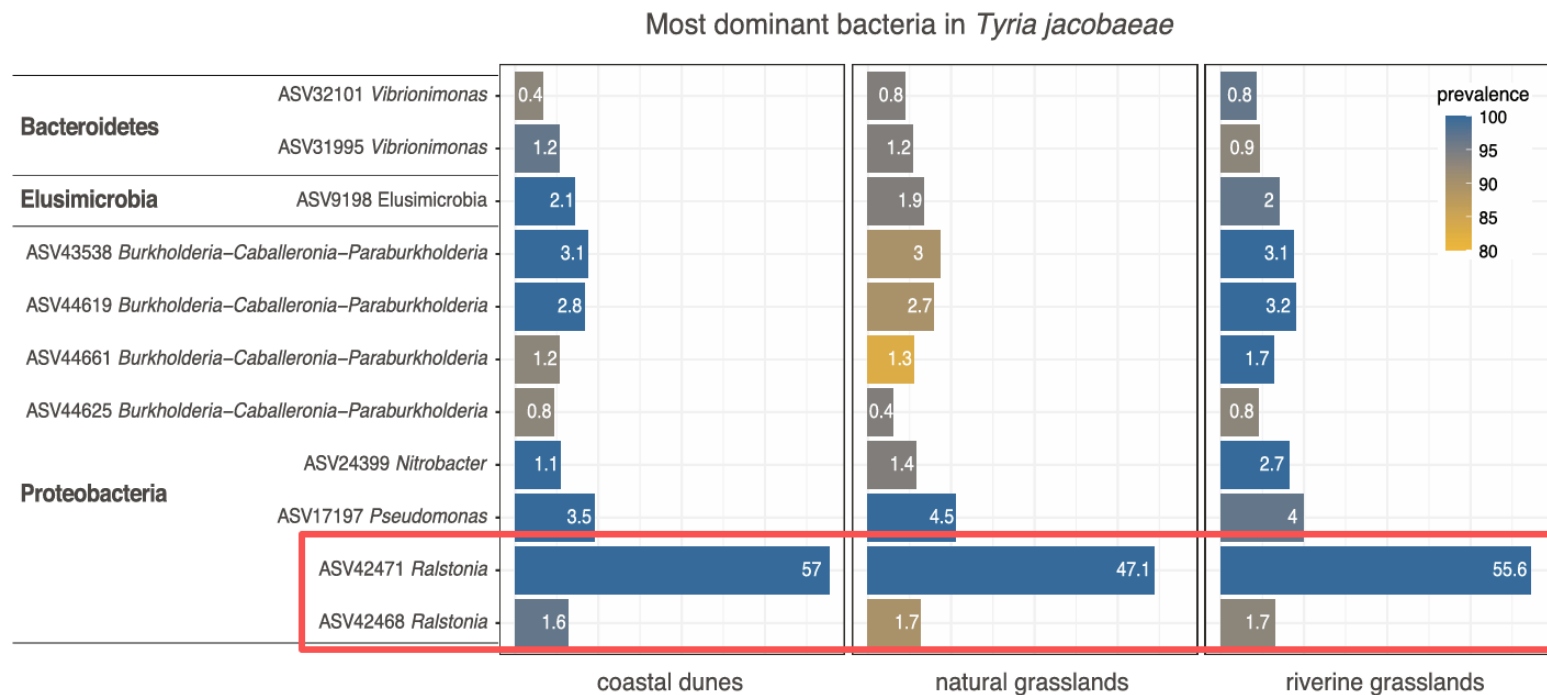


Background and Scientific Questions

- Whether Lepidoptera harbor a conserved core gut microbiome has long been debated;
- The soil microbial pool, the largest microbial reservoir on Earth, is a likely environmental source of gut bacteria in folivorous larvae;
- **Scientific question: Can soil-soiled bacteria broadly colonize the gut of leaf-feeding larvae, and how do they shape host fitness?**



(Shao et al., 2024. Annu Rev Entomol)



(Fernandes Gomes et al., 2020. Anim Microbiome)



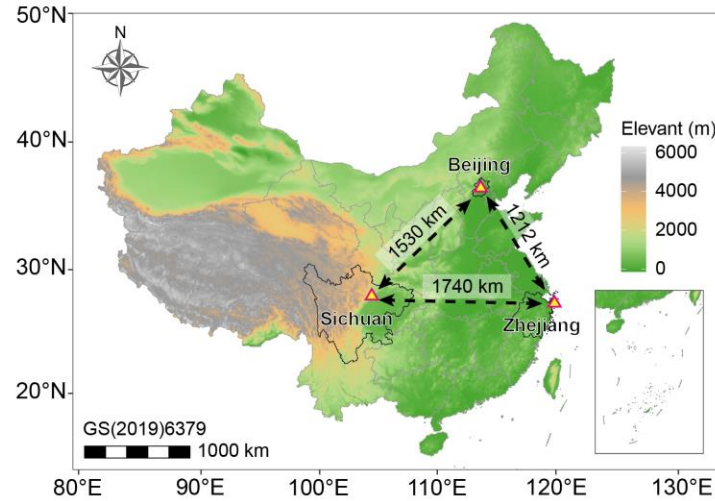
Study Design and Sampling Strategy

➤ Sites:

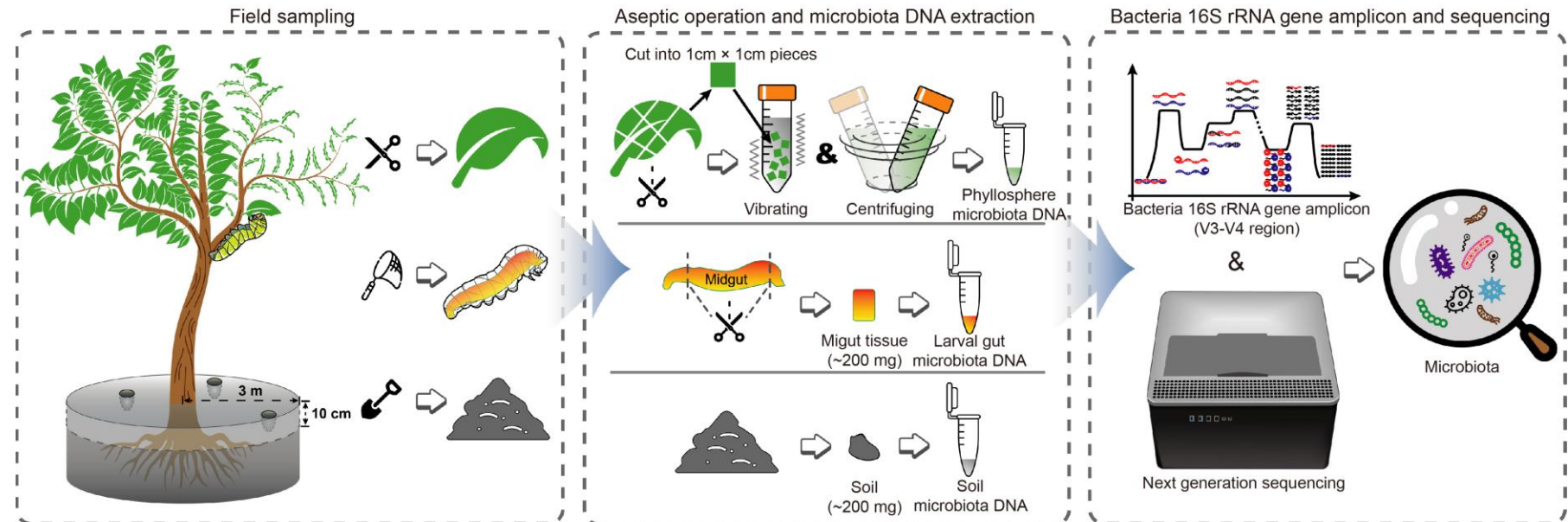
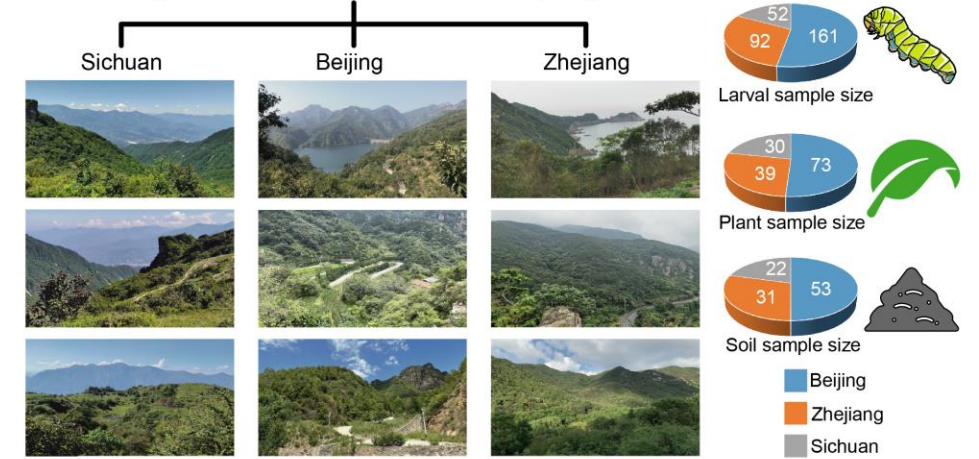
Beijing,
Sichuan,
Zhejiang.

➤ Samples:

305 folivorous larvae,
142 plant samples,
106 soil samples.



The ecological environment in the sampling fields

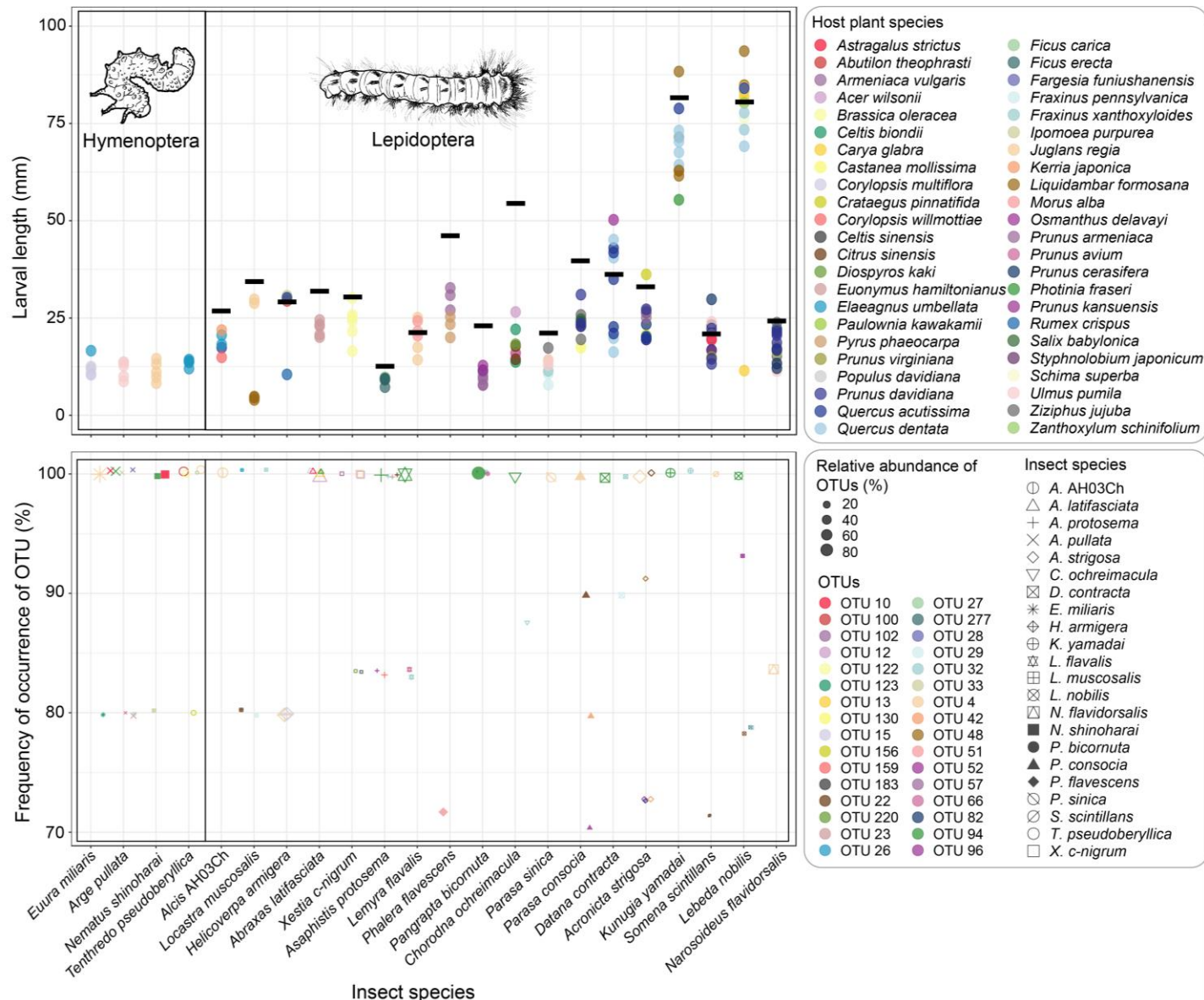




Results

Two generalist bacteria -- *Delftia* sp. and *Ralstonia insidiosa* -- broadly colonize the larval gut

- ◆ 32 high-frequency bacterial OTUs were recovered from the gut of 22 larval species.
- ◆ OTU 4 (Burkholderiales, *Delftia* sp.) and OTU 94 (Burkholderiales, *Ralstonia insidiosa*) dominated the gut microbiota of most larval species.
- ◆ *Delftia* sp. was detected in 10 larval species with a mean relative abundance of 38.96%; *R. insidiosa* occurred in another 10 species with a mean relative abundance of 34.44%.



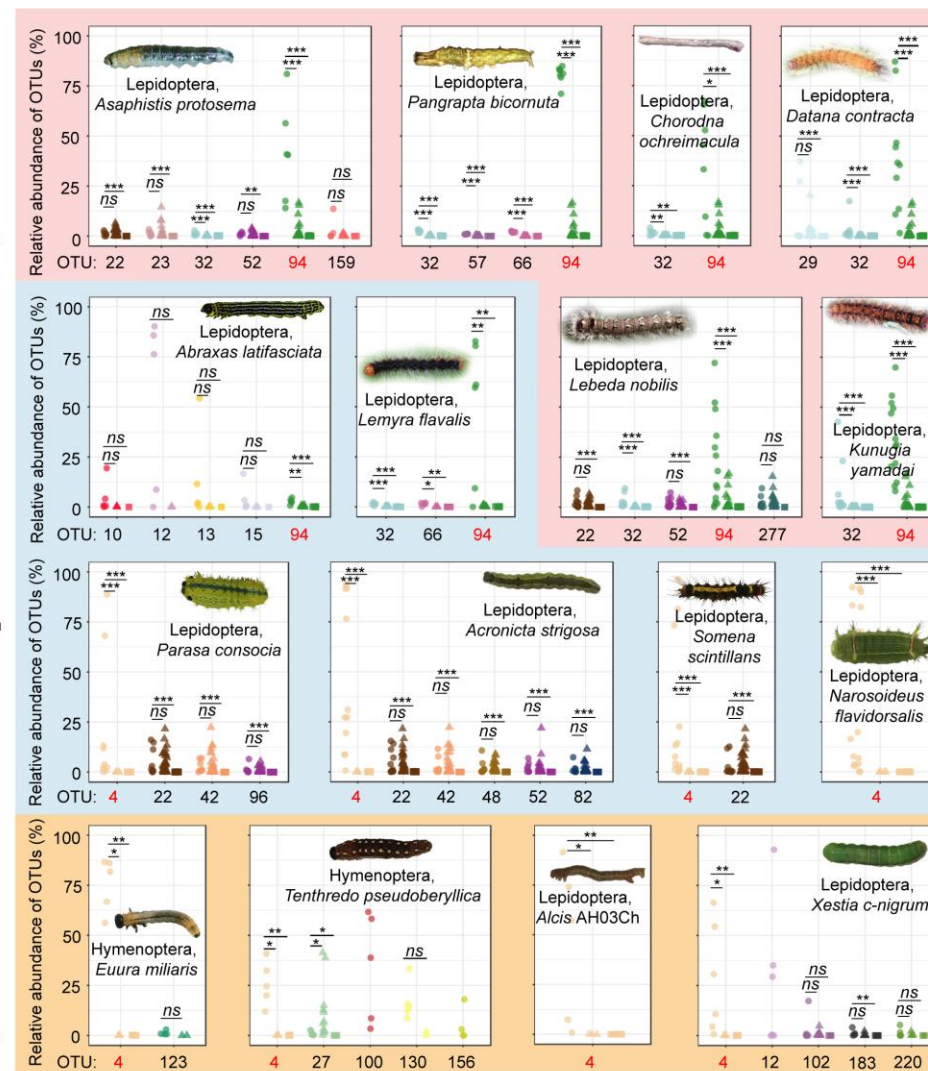
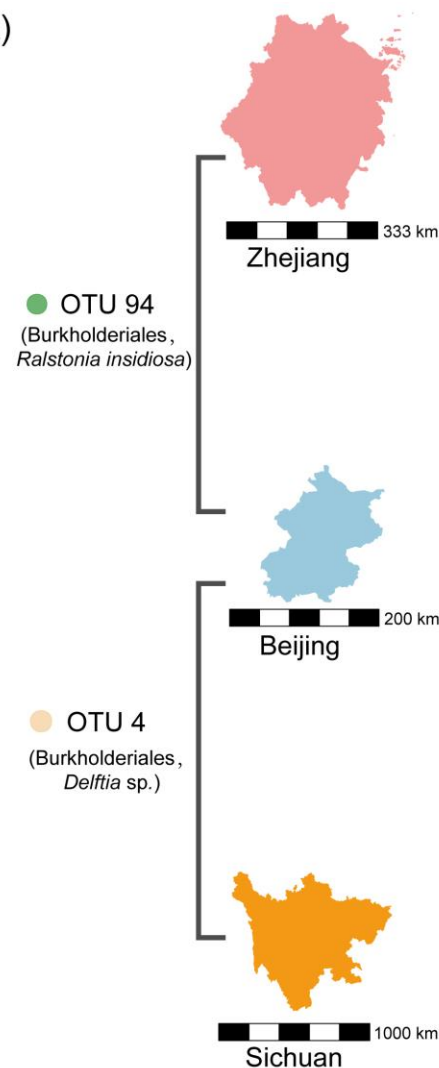


Results

Two generalist bacteria -- *Delftia* sp. and *Ralstonia insidiosa* -- broadly colonize the larval gut

- ◆ Both bacteria were significantly enriched in the gut of 16 larval species.
- ◆ Across the study, 97.92% of larval species and 90.16% of individuals were tightly associated with one of the two generalist bacteria.

(A)



OTUs

- OTU 10
- OTU 100
- OTU 102
- OTU 12
- OTU 123
- OTU 13
- OTU 130
- OTU 15
- OTU 156
- OTU 159
- OTU 183
- OTU 22
- OTU 220
- OTU 23
- OTU 27
- OTU 277
- OTU 29
- OTU 32
- OTU 4
- OTU 42
- OTU 48
- OTU 52
- OTU 57
- OTU 66
- OTU 82
- OTU 94
- OTU 96

Sample type

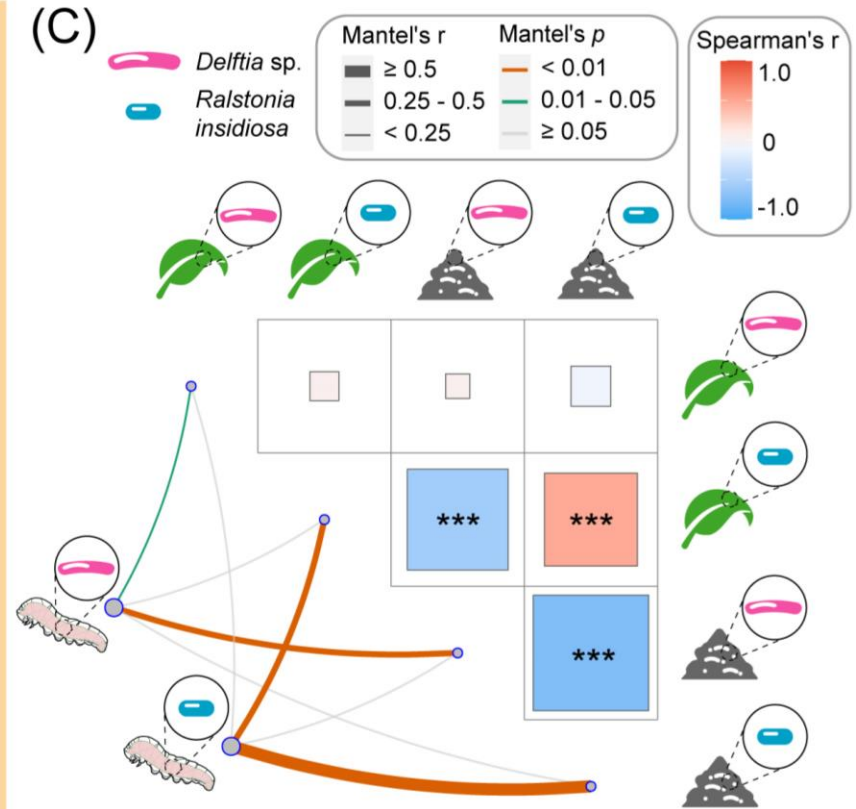
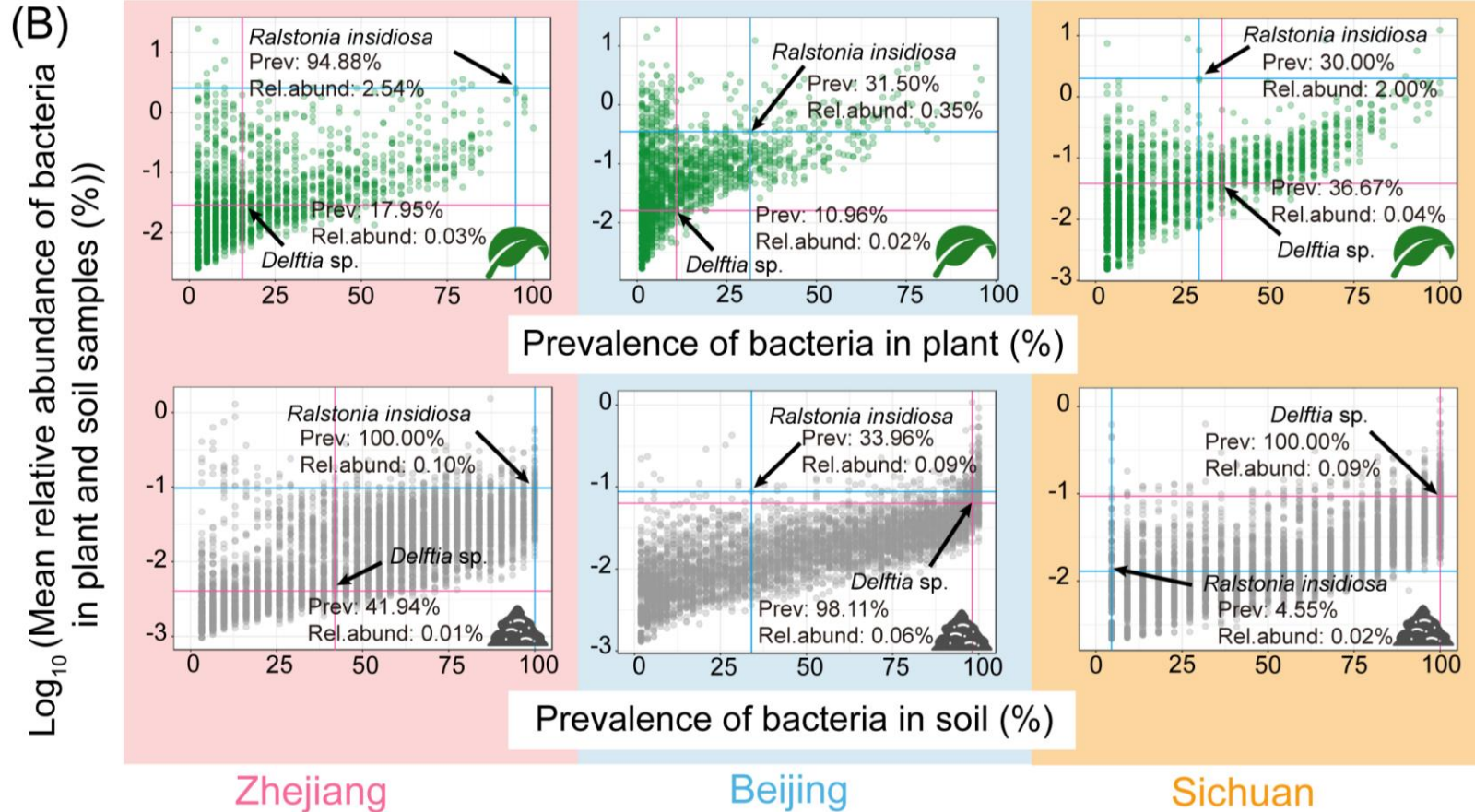
- Larval gut
- Plant
- Soil



Results

Sources of the Two Generalist Bacteria

- ◆ At least one generalist bacterium was detected in nearly all soil samples.
- ◆ The generalist bacteria in larval guts showed significantly stronger correlations with their counterparts in soil samples than with those in phyllosphere samples.

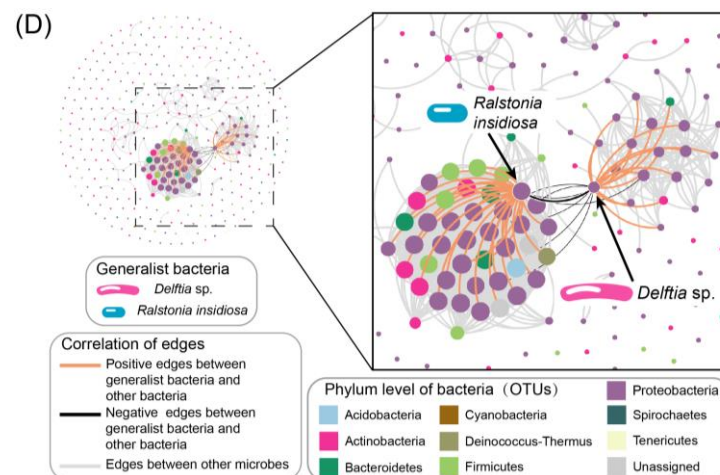




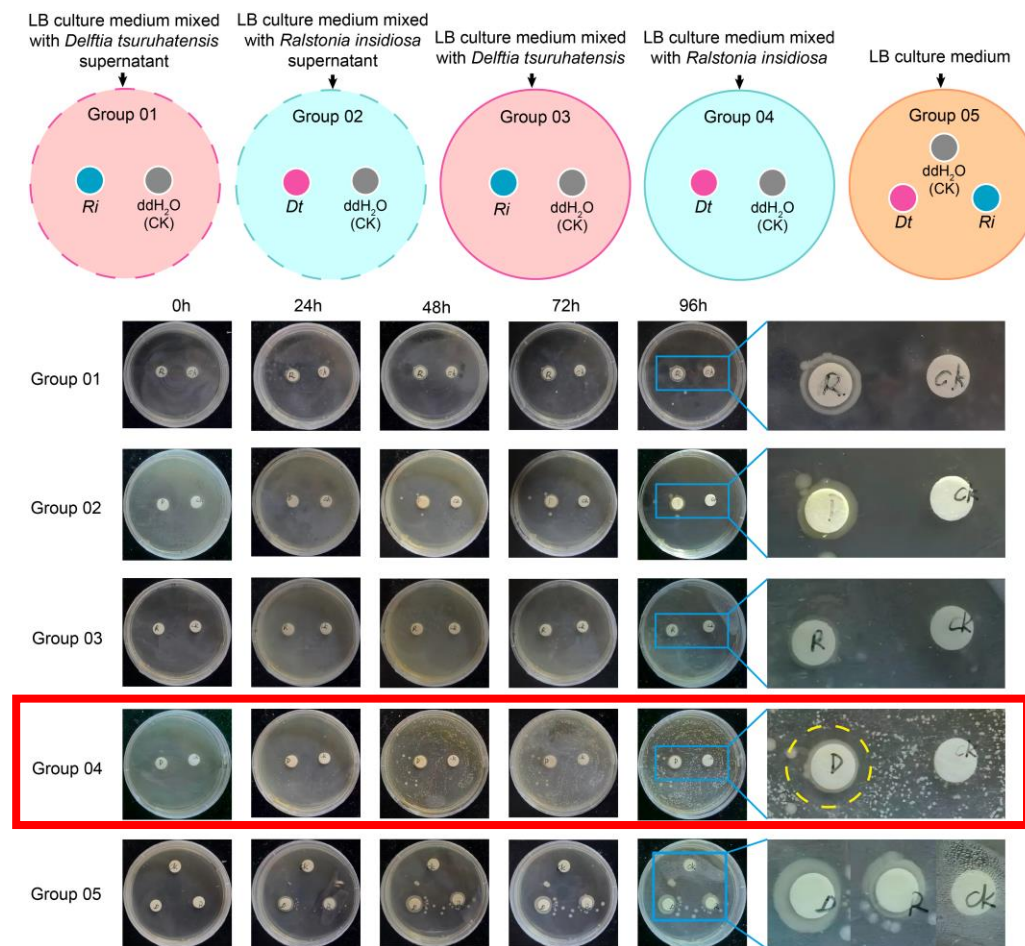
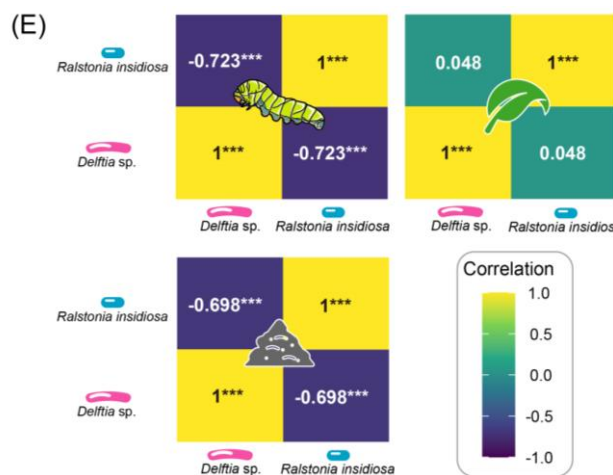
Results

Mutual exclusion between the two generalist bacteria

- ◆ A co-occurrence network across all larval samples revealed a significant negative correlation between *R. insidiosa* and *Delftia* sp. ($r = -0.723$, $p < 0.001$).



- ◆ The inhibition zone assay also revealed antagonism between the two bacteria: the presence of *Delftia* sp. significantly inhibited the growth of *R. insidiosa*.



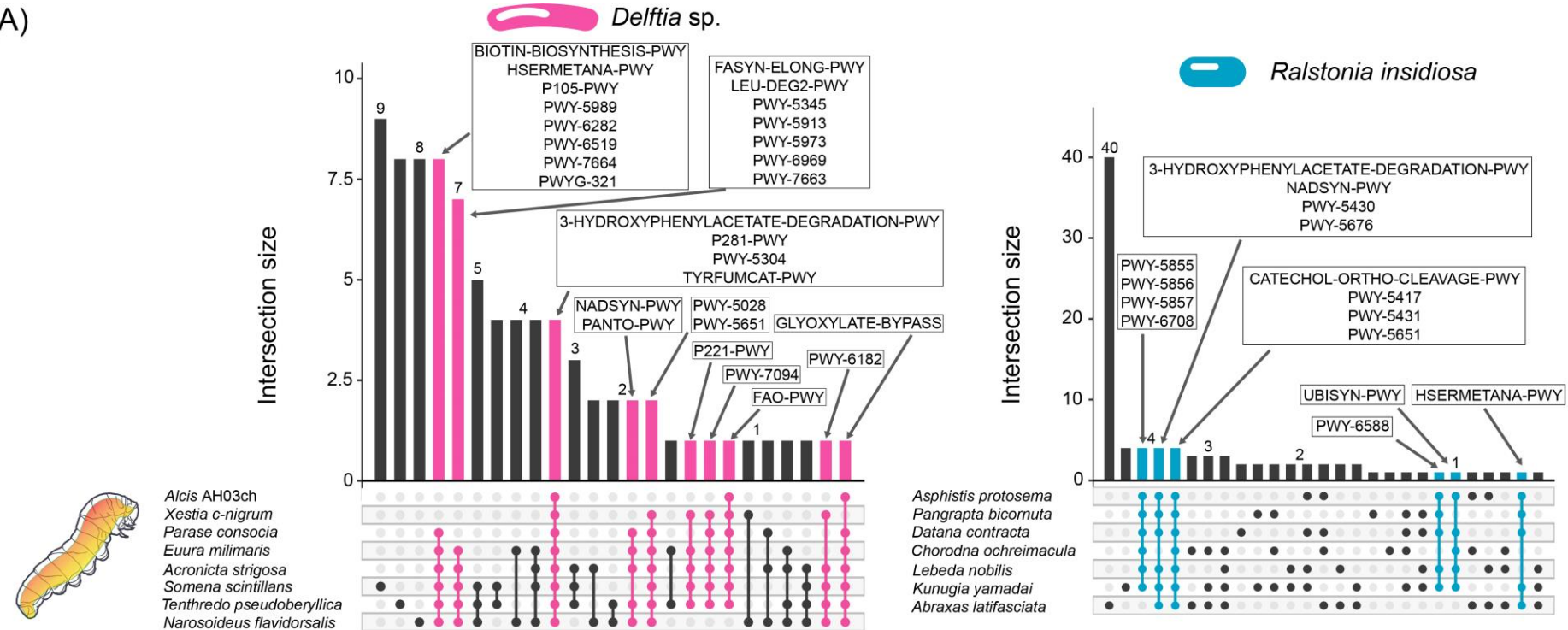


Results

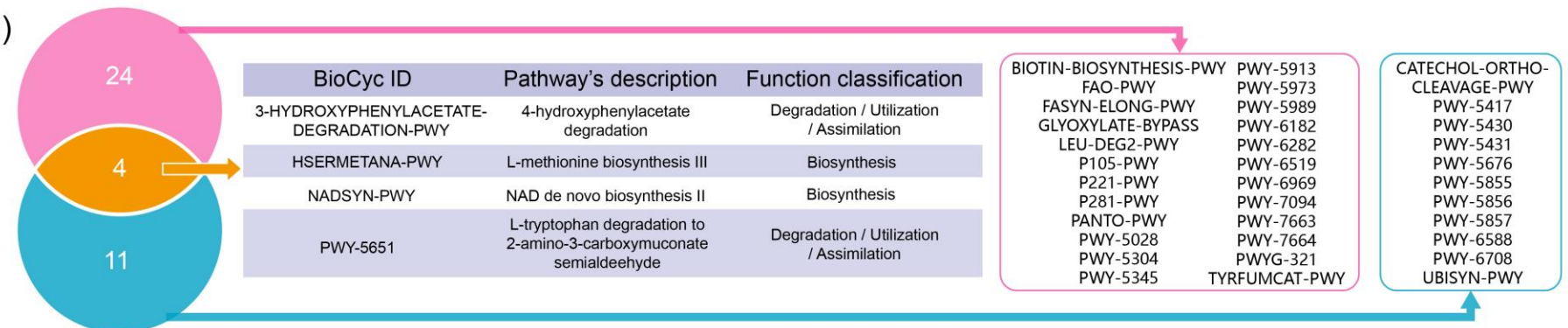
Competitive advantages of the two generalist bacteria in the larval gut

- Of the four conserved core metabolic pathways shared by the two bacteria, three are linked to energy metabolism.
- Relative to other gut bacteria, both broad-host taxa show higher energy-metabolism efficiency in the larval gut, which may enhance their competitive ability for the gut niche.

(A)



(B)

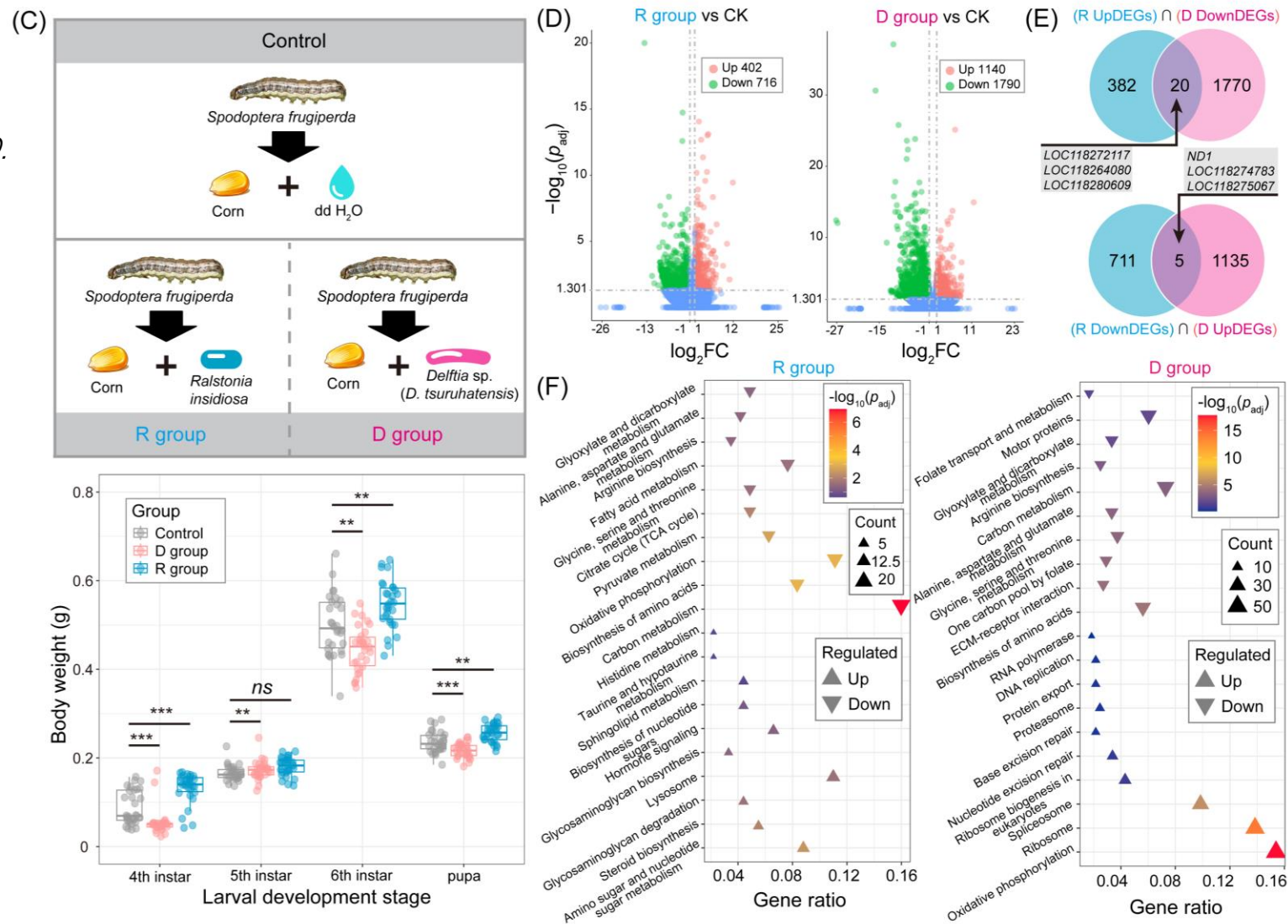




Results

Colonization of the generalists in folivorous larvae and their effects on the host

- ◆ *R. insidiosa* colonization increased larval body weight at both the sixth-instar and pupal stages (R group), whereas *Delftia* sp. (congeneric strain *D. tsuruhatensis*) reduced larval body weight at the sixth-instar and pupal stages (D group).
- ◆ In the R group, genes involved in aerobic respiration and protein digestion were downregulated, and pathways related to carbon metabolism and the TCA cycle were broadly suppressed, indicating that energy was conserved for growth, resulting in increased body weight. In the D group, oxidative phosphorylation was upregulated, but pathways such as amino acid biosynthesis were downregulated, suggesting that energy was diverted toward homeostatic maintenance rather than growth, resulting in reduced body weight.



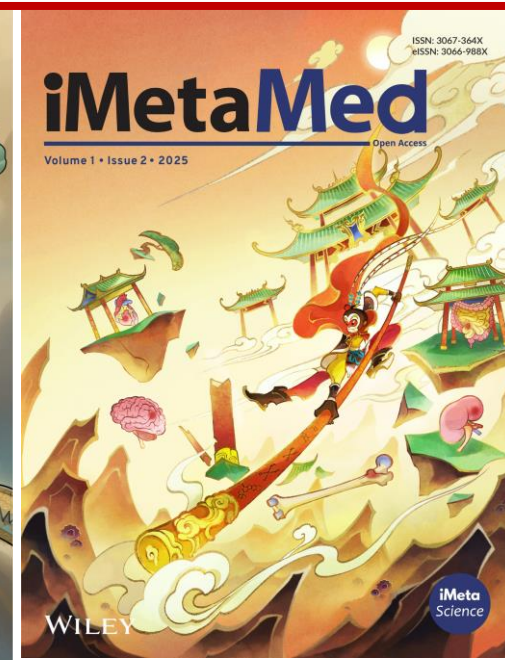
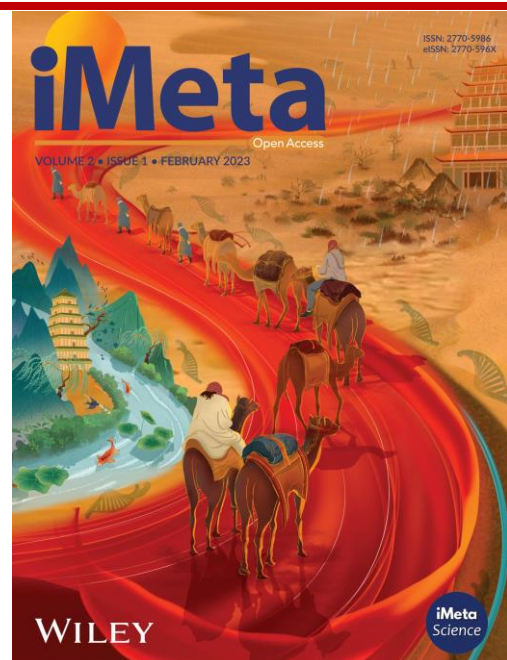


Summary

In summary, through extensive field sampling, this study revealed that the guts of diverse folivorous larvae are commonly enriched with two soil-derived generalist bacteria, *Delftia* sp. and *Ralstonia insidiosa*. Functional validation further demonstrated that *R. insidiosa* and the representative *Delftia* strain *D. tsuruhatensis* exert distinct effects on larval body weight and host metabolic pathways, suggesting that colonization by *Ralstonia* and *Delftia* may shape host growth and development in different directions. Together, these findings deepen our understanding of the complexity of insect–microbe interactions and have important implications for ecosystem management and agricultural production.

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