Supplementary Information

Gut microbiomes of cycad-feeding insects tolerant to β-methylamino-L-alanine (BMAA) are rich in siderophore biosynthesis

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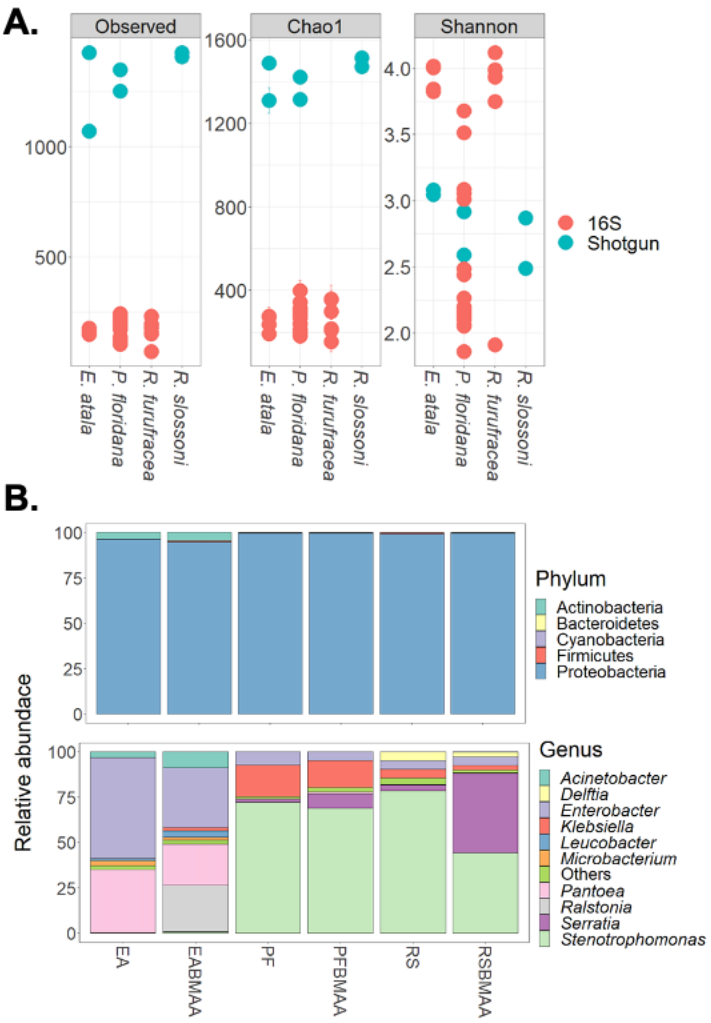
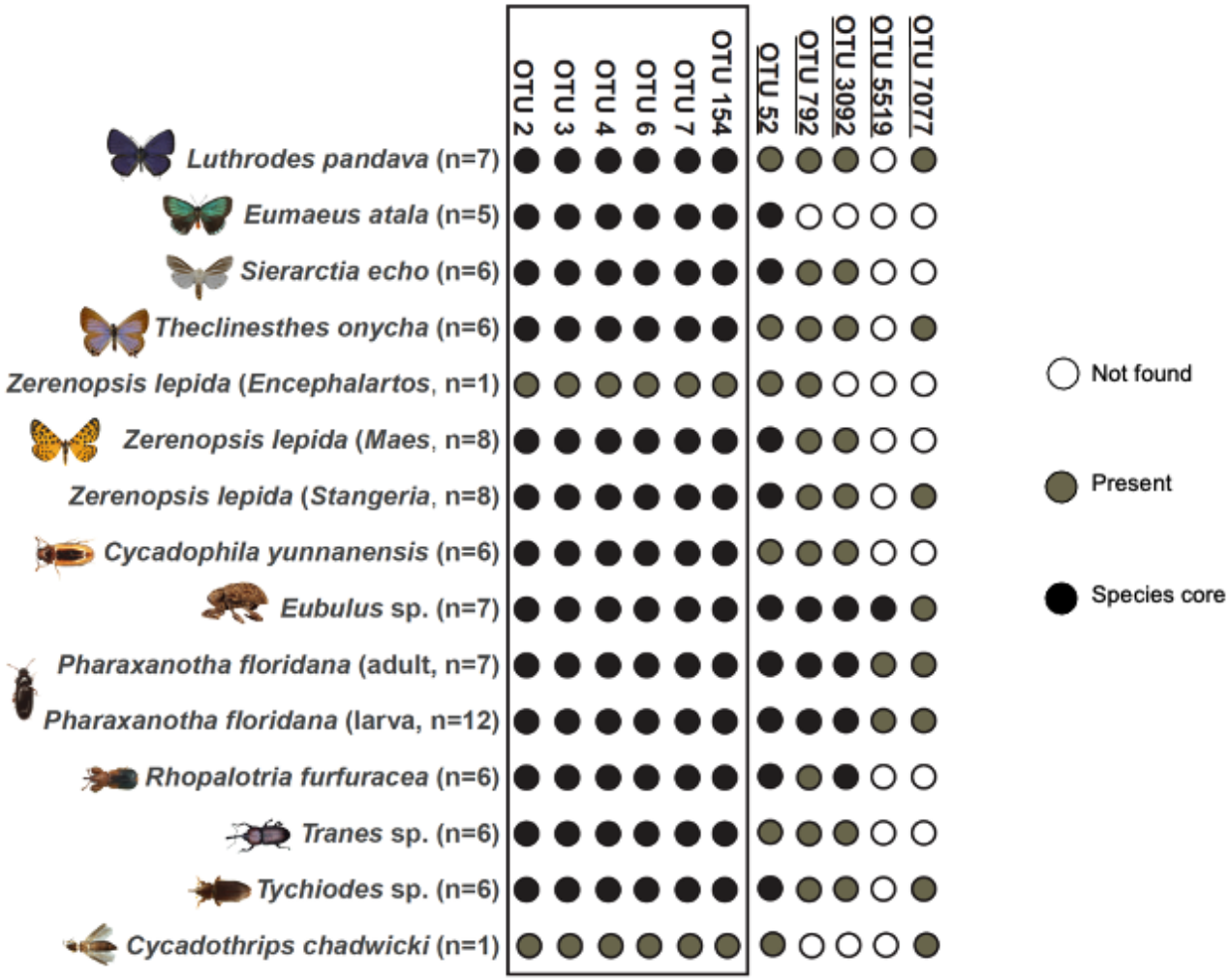
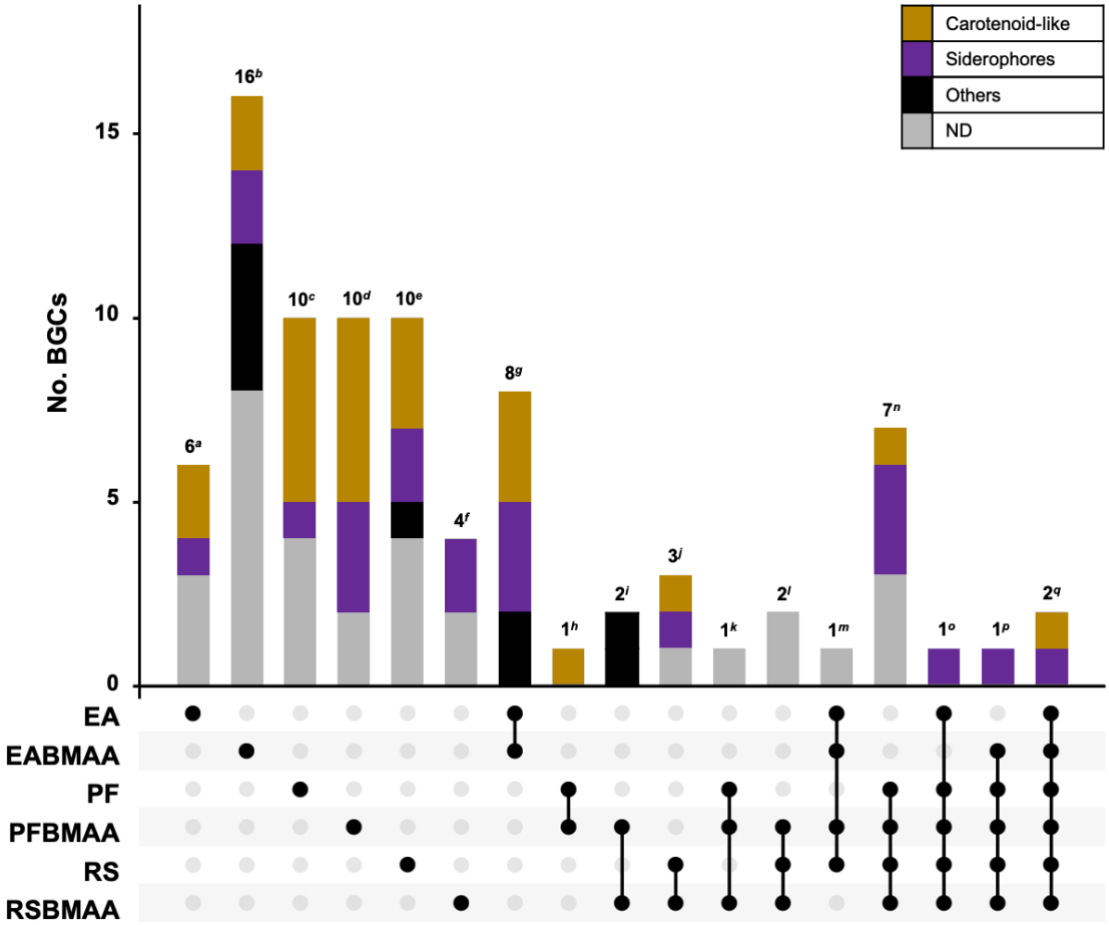


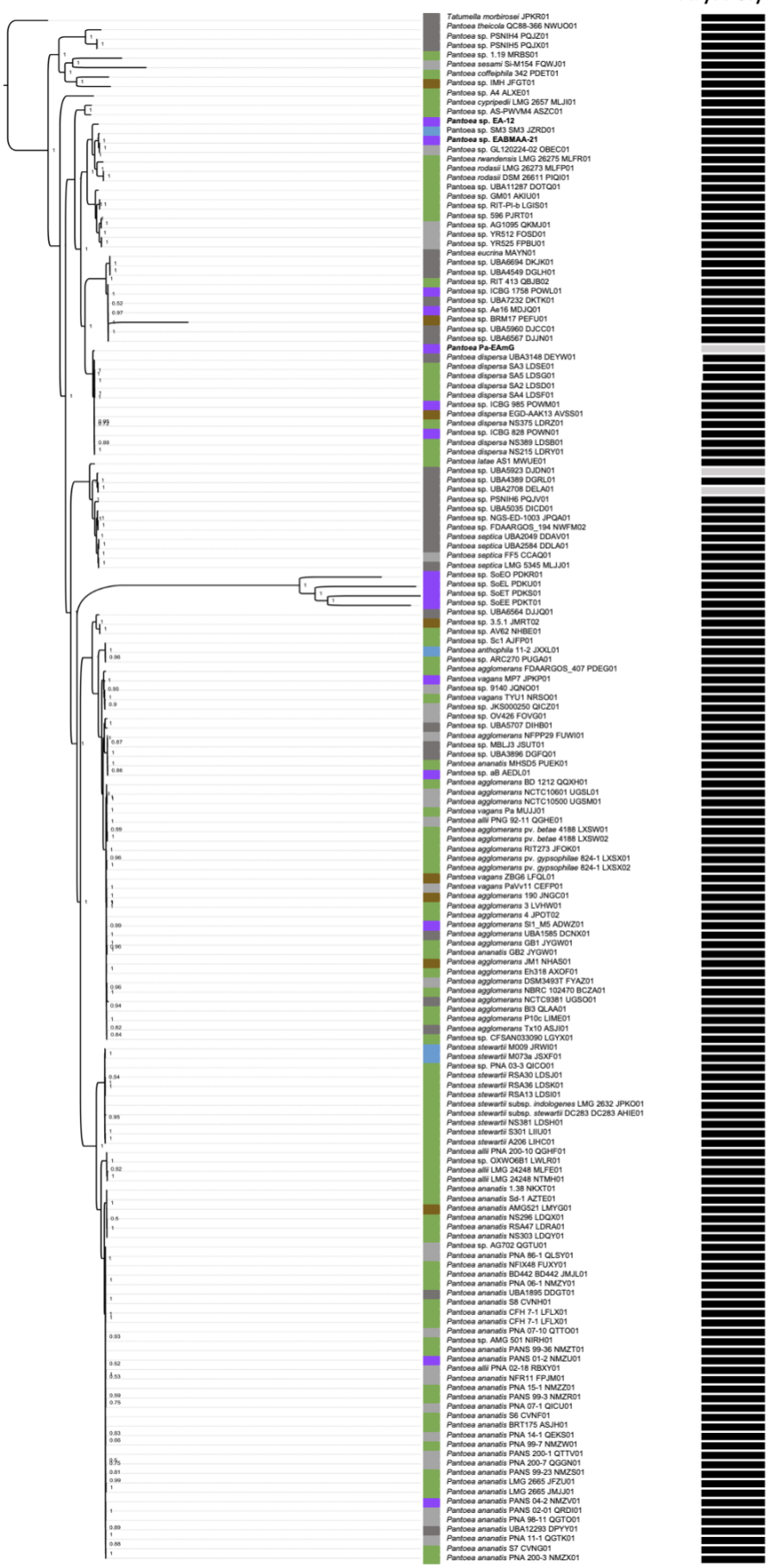
Supplementary Figure S1. Taxonomic analysis of shotgun metagenomes. A. Alpha diversity comparison of 16S and shotgun metagenomes. **B.** Relative abundance of taxonomically classified and filtered reads in each shotgun metagenome assigned to the phylum and genus level, and the 10 most abundant genera. EA, *Eumaeus atala* (BMAA +/-); PF, *Pharaxonotha floridana* (BMAA +/-); RS, *Rhopalotria slossoni* (BMAA +/-).



Supplementary Figure S2. Metagenomics and genomics data integration reveal keystone taxa. Black circles indicate OTUs belonging to an insect species' core, grey circles represent non-core OTUs present in some individuals within a species/grouping, and white circles indicate OTUs that were not found in any individual within a species/grouping. Core taxa are shown within a box and semi conserved taxa shown underlined without a box.



Supplementary Figure S3. Presence and absence BGC plot. 85 complete and non-redundant BGCs identified in the six metagenomes obtained from the co-cultures were used to construct the BGC plot. Three specific BGCs, one turnebactin-like BGC from the catechol-type siderophore category and one carotenoid-like aryl polyene, were found to be present in all the metagenomes. EA, *Eumaeus atala* (BMAA +/-); PF, *Pharaxonotha floridana* (BMAA +/-); RS, *Rhopalotria slossoni* (BMAA +/-). Superscript labels indicate the number of complete and non-redundant BGCs detected in the metagenome sequences. Detail information of these BGCs is available in Table S12.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Pantoea sp. PSNIH4 PQJZ01
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Pantoea sp. 1.19 MRBS01
Pantoea sesami Si-M154 FQWJ01
Pantoea coffeiphila 342 PDET01
Pantoea sp. IMH JFGT01
Pantoea sp. A4 ALXE01
Pantoea cypripedii LMG 2657 MLJI01
Pantoea sp. AS-PWVM4 ASZC01
Pantoea sp. EA-12
Pantoea sp. SM3 SM3 JZRD01
Pantoea sp. EABMAA-21
Pantoea sp. GL120224-02 OBEC01
Pantoea rwandensis LMG 26275 MLFR01
Pantoea rodasii LMG 26273 MLFP01
Pantoea rodasii DSM 26611 PIQI01
Pantoea sp. UBA11287 DOTQ01
Pantoea sp. GM01 AKIU01
Pantoea sp. RIT-PI-b LGIS01
Pantoea sp. 596 PJRT01
Pantoea sp. AG1095 QKMJ01
Pantoea sp. YR512 FOSD01
Pantoea sp. YR525 FPBU01
Pantoea eucrina MAYN01
Pantoea sp. UBA6694 DKJK01
Pantoea sp. UBA4549 DGLH01
Pantoea sp. RIT 413 QBJB02
Pantoea sp. ICBG 1758 POWL01
Pantoea sp. UBA7232 DKTK01
Pantoea sp. Ae16 MDJQ01
Pantoea sp. BRM17 PEFU01
Pantoea sp. UBA5960 DJCC01
Pantoea sp. UBA6567 DJJN01
Pantoea Pe-EAm6
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Pantoea dispersa SA3 LDSE01
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Pantoea agglomerans NCTC10500 UGSM01
Pantoea vagans Pa MUJJ01
Pantoea allii PNG 92-11 QGHE01
Pantoea agglomerans pv. betae 4188 LXSW01
Pantoea agglomerans pv. betae 4188 LXSW02
Pantoea agglomerans RIT273 JFOK01
Pantoea agglomerans pv. gypsophilae 824-1 LXSX01
Pantoea agglomerans pv. gypsophilae 824-1 LXSX02
Pantoea vagans ZBG6 LFQL01
Pantoea vagans PaVv11 CEFP01
Pantoea agglomerans 190 JNGC01
Pantoea agglomerans 3 LVHW01
Pantoea agglomerans 4 JPOT02
Pantoea agglomerans St1_M5 ADWZ01
Pantoea agglomerans UBA1585 DCNX01
Pantoea agglomerans GB1 JYGW01
Pantoea ananatis GB2 JYGW01
Pantoea agglomerans JM1 NHAS01
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Pantoea agglomerans Tx10 ASJI01
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Pantoea stewartii RSA13 LDSI01
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Pantoea stewartii subsp. stewartii DC283 DC283 AHIE01
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Pantoea stewartii A206 LIHC01
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Pantoea ananatis S7 CVNG01
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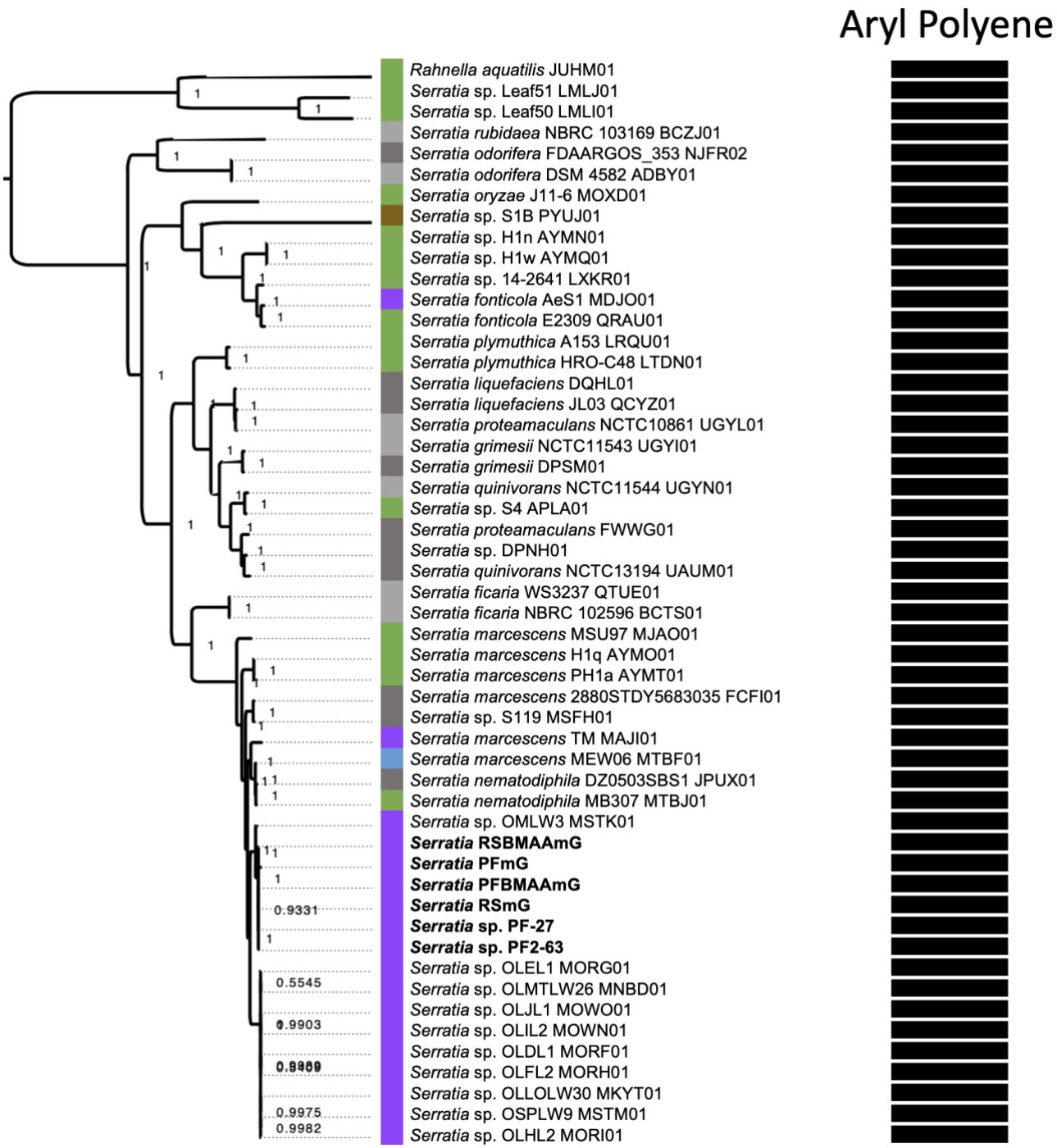
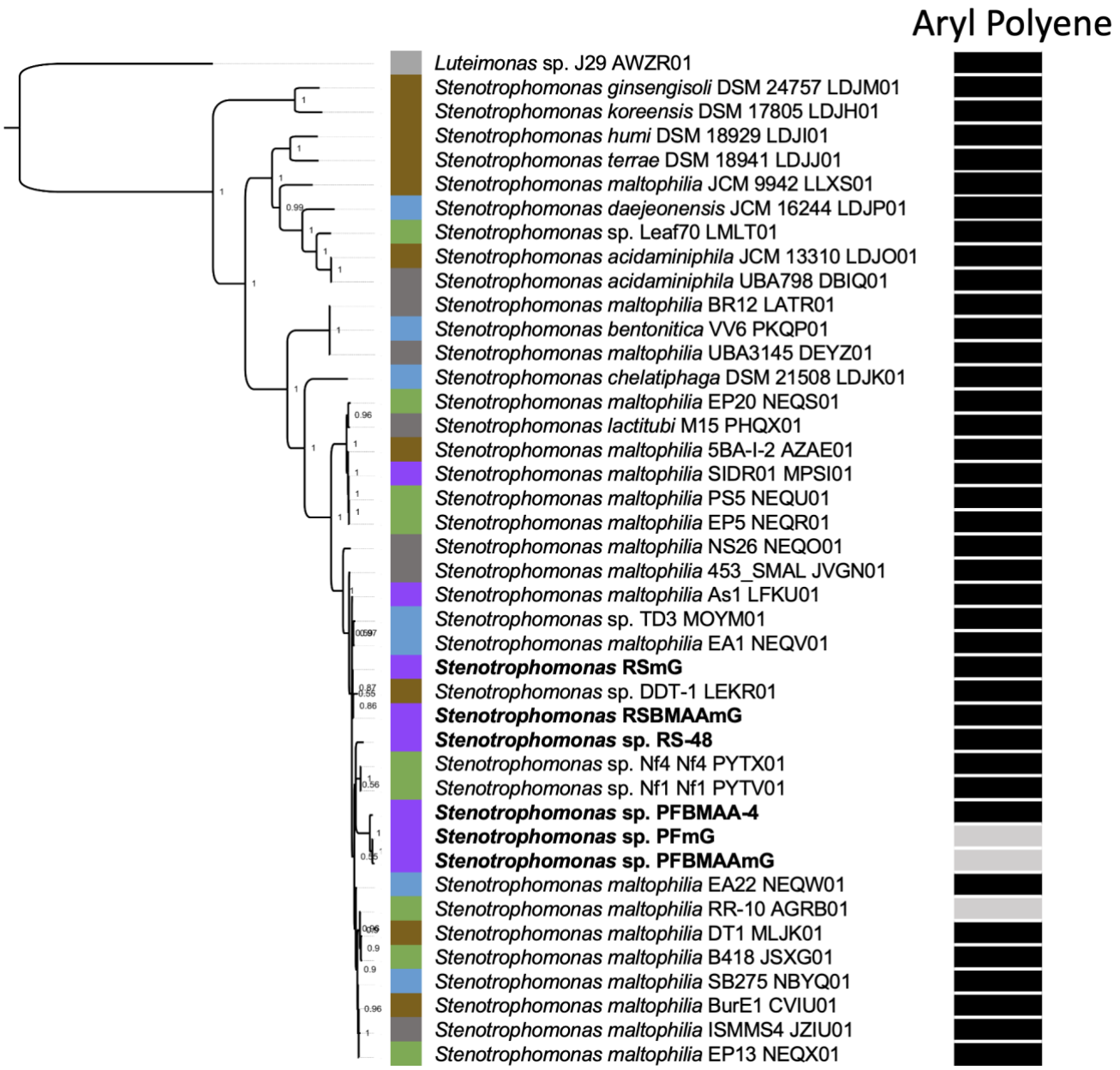
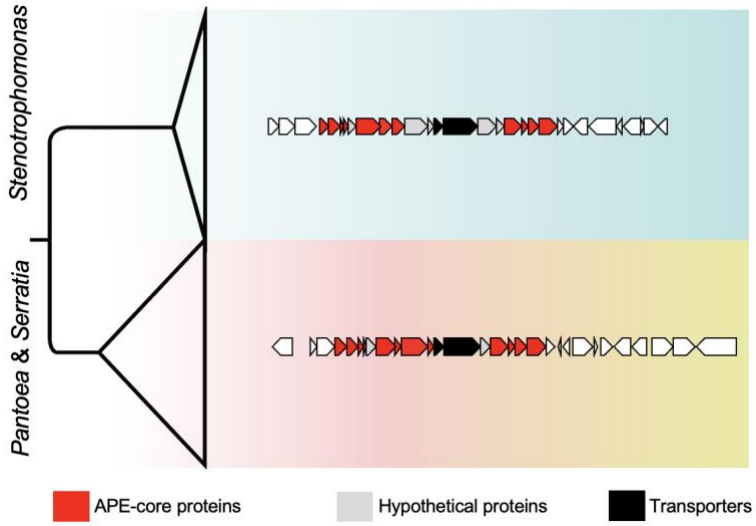
Supplementary Figure S4. Full *Pantoea* phylogenetic tree of representative strains, MAGs and isolated strains from the co-cultures. 168 *Pantoea* genomes were used to reconstruct this phylogeny using the core proteome composed of 64 proteins (Table S4 and S7). Habitats for each species are indicated with colored bullets. Purple = insects, Green = plants, Brown = soil, Blue = water, Dark gray = Other, and Light gray = Not determined. The incidence of the aryl polyene BGCs is shown as presence (black bars) or absence (light gray bars). Phylogenetic tree was constructed using a Bayesian method, employing a mixed substitution model over the course of 100,000 generations.



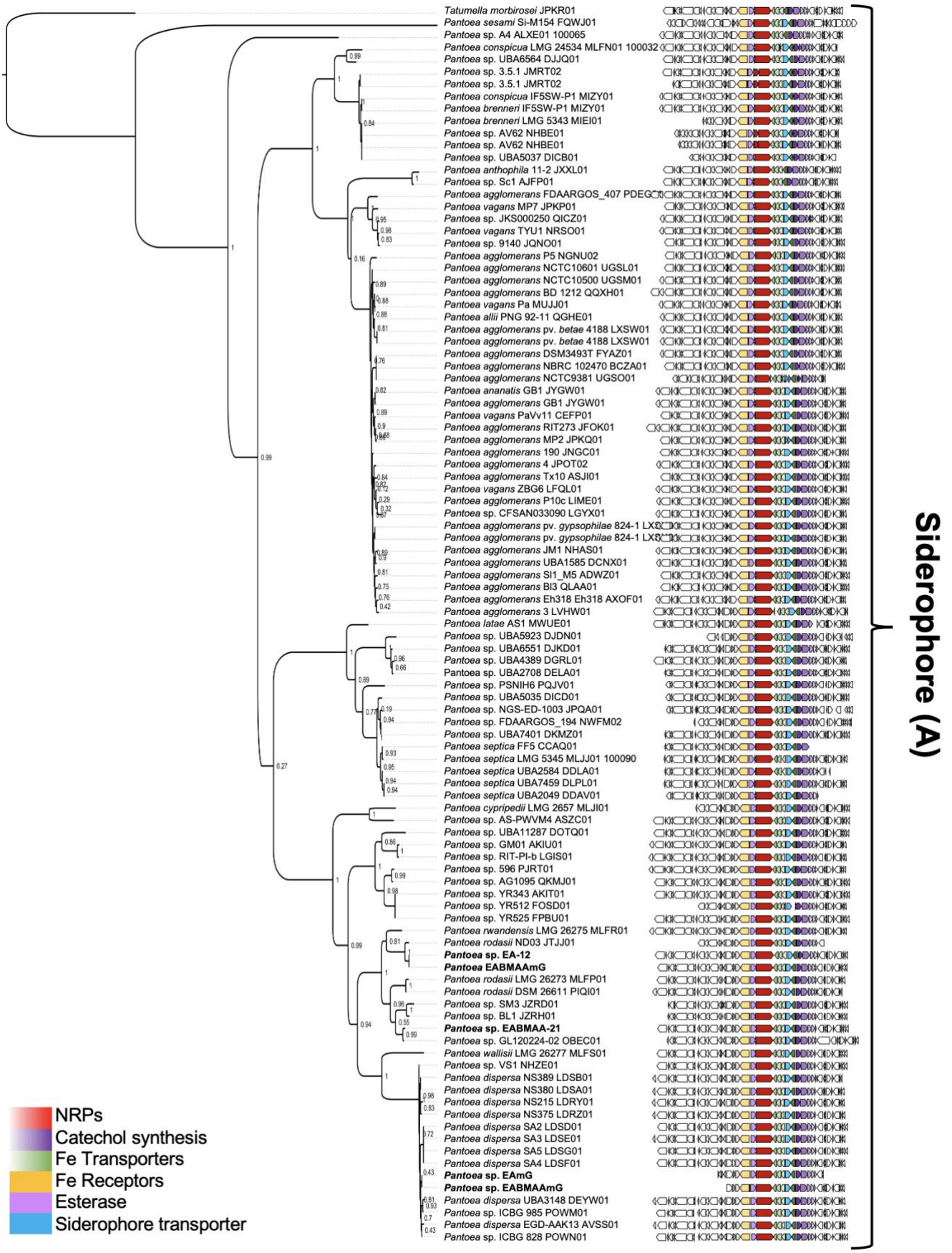
Supplementary Figure S5. Full *Serratia* phylogenetic tree of representative strains, MAGs and isolated strains from the co-cultures. 51 *Serratia* genomes were used to reconstruct this phylogeny using the core proteome composed of 712 proteins (Table S5 and S8). Habitats for each species are indicated with colored bullets. Purple = insects, Green = plants, Brown = soil, Blue = water, Dark gray = Other, and Light gray = Not determined. The incidence of the aryl polyene BGCs is shown as present in all the *Serratia* genomes (Black bars). Phylogenetic tree was constructed using a Bayesian method, employing a mixed substitution model over the course of 100,000 generations.



Supplementary Figure S6. Full *Stenotrophomonas* phylogenetic tree of representative strains, MAGs and isolated strains from the co-cultures. 41 *Serratia* genomes were used to reconstruct this phylogeny using the core proteome composed of 39 proteins (Table S3 and S6). Habitats for each species are indicated with colored bullets. Purple = insects, Green = plants, Brown = soil, Blue = water, Dark gray = Other, and Light gray = Not determined. The incidence of the aryl polyene BGCs is shown as presence (black bars) or absence (light gray bars). Phylogenetic tree was constructed using a Bayesian method, employing a mixed substitution model over the course of 100,000 generations.



Supplementary Figure S7. Aryl Polyene BGCs phylogeny of *Serratia, Pantoea,* and *Stenotrophomonas* species. 254 Aryl polyene BGCs from S*erratia*, *Pantoea*, and *Stenotrophomonas* genomes were used to reconstruct this phylogeny. Aryl polyene BGCs are highly conserved in all three bacterial genera. Phylogenetic tree was constructed using a Bayesian method, employing a mixed substitution model over the course of 100,000 generations.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Pantoea sp. UBA6564 DJJQ01
Pantoea sp. 3.5.1 JMRT02
Pantoea sp. 3.5.1 JMRT02
Pantoea conspicua IF5SW-P1 MIZY01
Pantoea brenneri IF5SW-P1 MIZY01
Pantoea brenneri LMG 5343 MIEI01
Pantoea sp. AV62 NHBE01
Pantoea sp. AV62 NHBE01
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Pantoea vagans Pa MUJJ01
Pantoea allii PNG 92-11 QGHE01
Pantoea agglomerans pv. betae 4188 LXSW01
Pantoea agglomerans pv. betae 4188 LXSW01
Pantoea agglomerans DSM3493T FYAZ01
Pantoea agglomerans NBRC 102470 BCZA01
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Pantoea vagans ZBG6 LFQL01
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Pantoea sp. CFSAN033090 LGYX01
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Pantoea agglomerans pv. gypsophilae 824-1 LX
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Pantoea agglomerans UBA1585 DCNX01
Pantoea agglomerans SI1_M5 ADWZ01
Pantoea agglomerans BI3 QLAA01
Pantoea agglomerans Eh318 Eh318 AXOF01
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Pantoea latae AS1 MWUE01
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Pantoea sp. BL1 JZRH01
Pantoea sp. EABMAA-21
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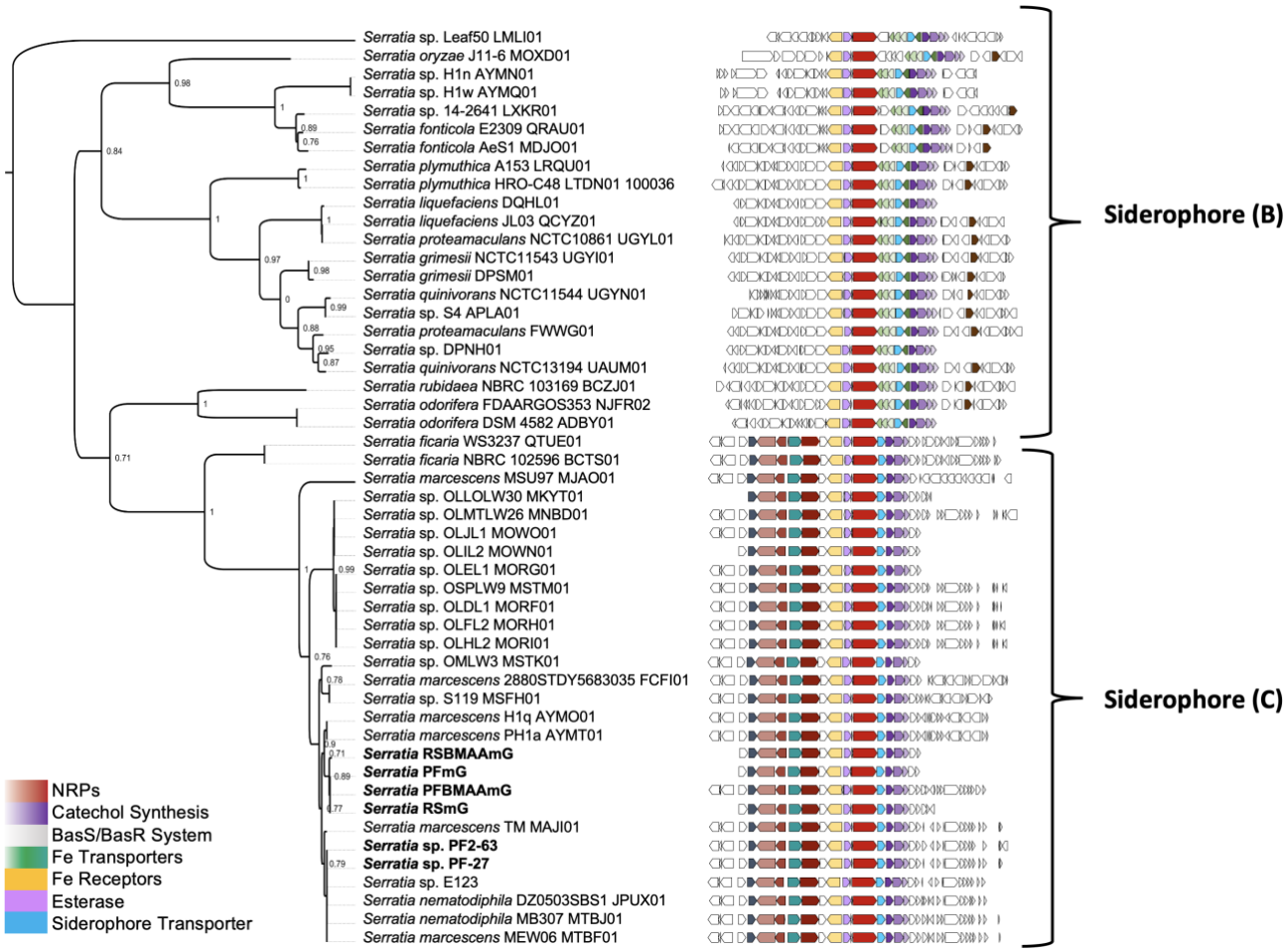
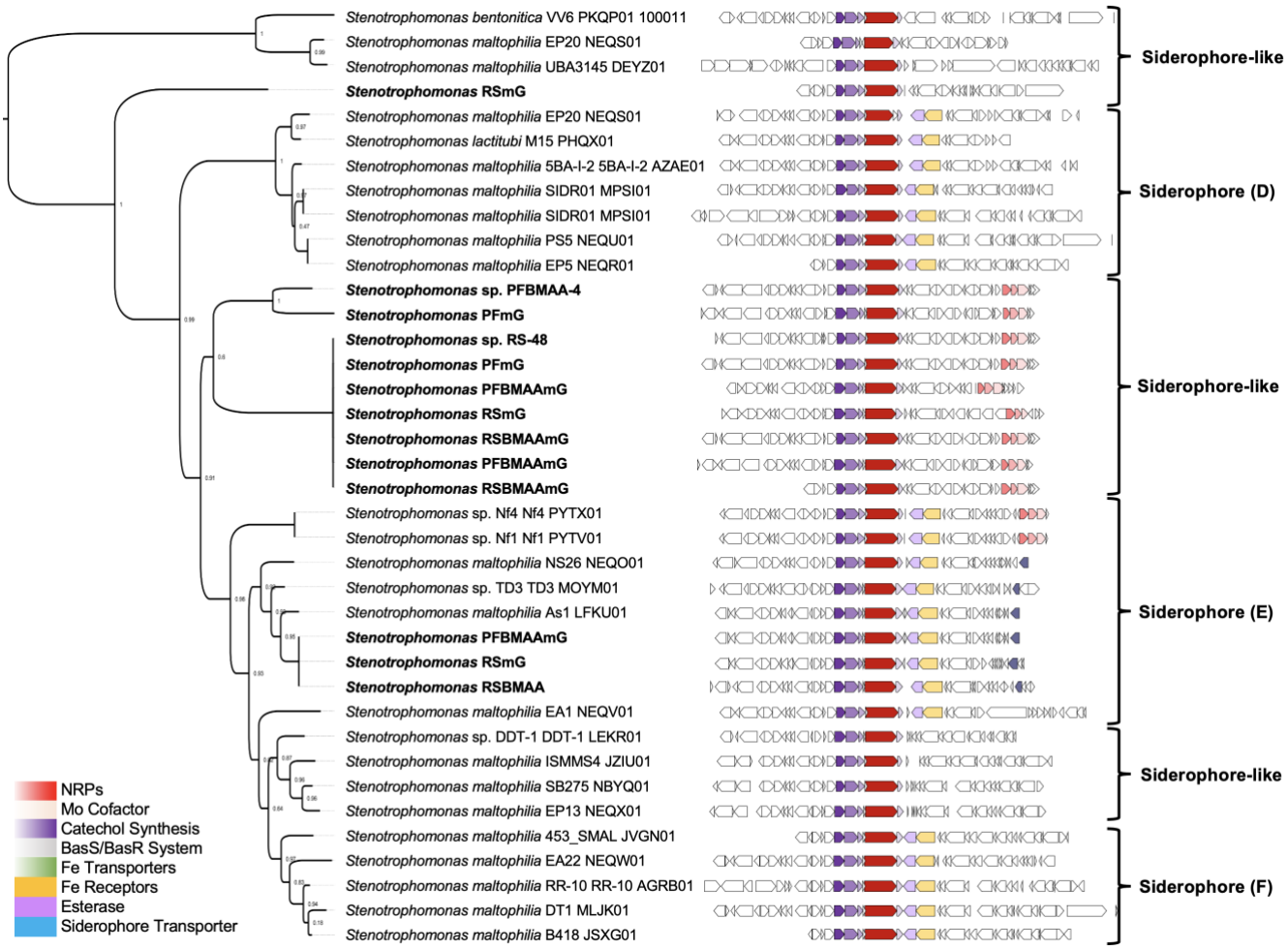
Pantoea wallisii LMG 26277 MLFS01
Pantoea sp. VS1 NHZE01
Pantoea dispersa NS389 DLSB01
Pantoea dispersa NS380 LDSA01
Pantoea dispersa NS215 LDRY01
Pantoea dispersa NS375 LDRZ01
Pantoea dispersa SA2 LDSD01
Pantoea dispersa SA3 LDSE01
Pantoea dispersa SA5 LDSG01
Pantoea dispersa SA4 LDSF01
Pantoea sp. EAmG
Pantoea sp. EABMAAmG
Pantoea dispersa UBA3148 DEYW01
Pantoea sp. ICBG 985 POWM01
Pantoea dispersa EGD-AAK13 AVSS01
Pantoea sp. ICBG 828 POWN01
NRPs
Catechol synthesis
Fe Transporters
Fe Receptors
Esterase
Siderophore transporter
Siderophore (A)

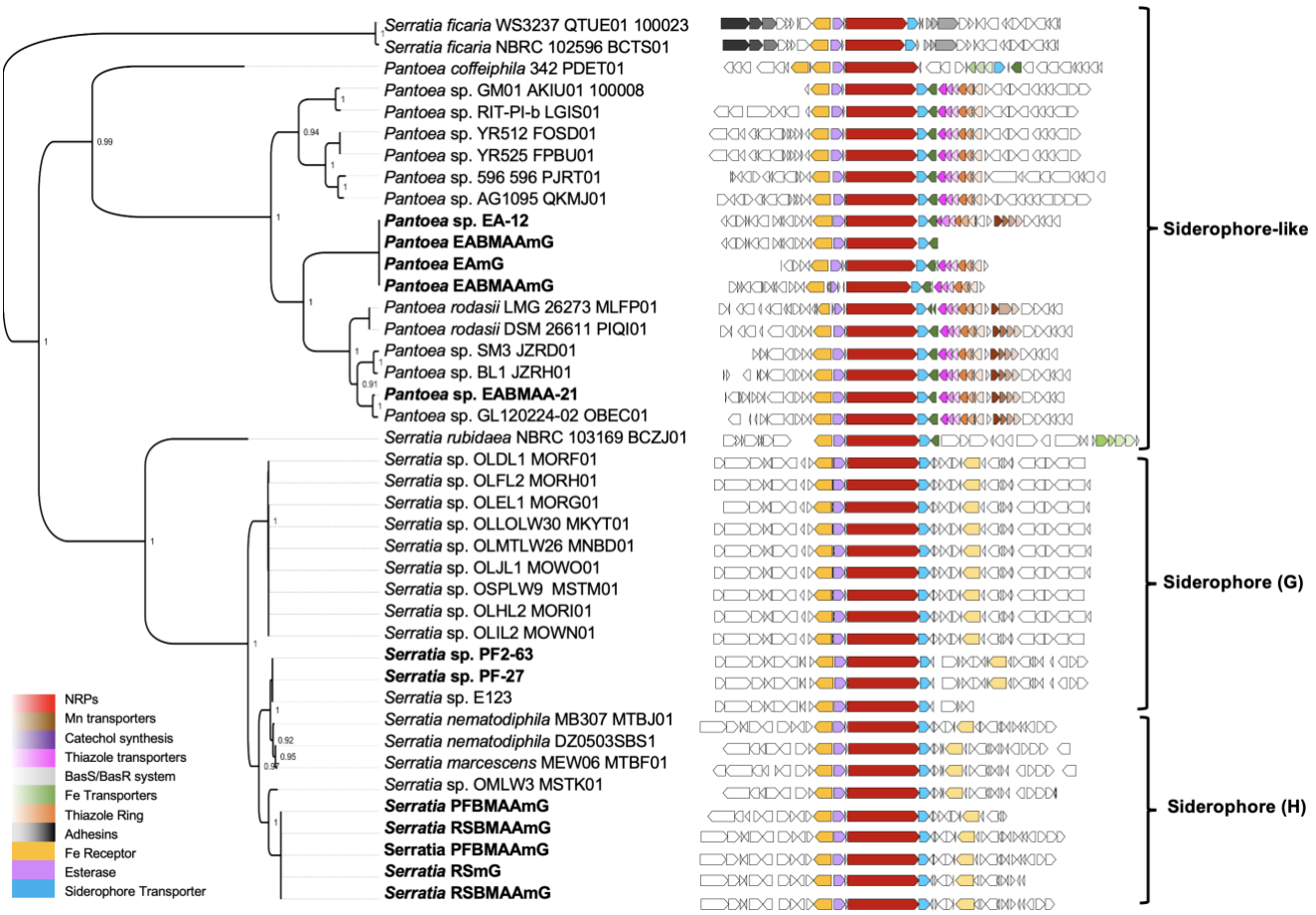
Supplementary Figure S8. Turnerbactin-like BGC phylogeny of *Pantoea*. 101 turnerbactin-like BGCs were used to reconstruct this phylogeny using the conserved proteins present in all BGCs. Genomic context visualization, as well as in-deep functional annotation of each BGC, reveal highly conservation in all the organisms. Phylogenetic tree was constructed using a Bayesian method, employing a mixed substitution model over the course of 100,000 generations.



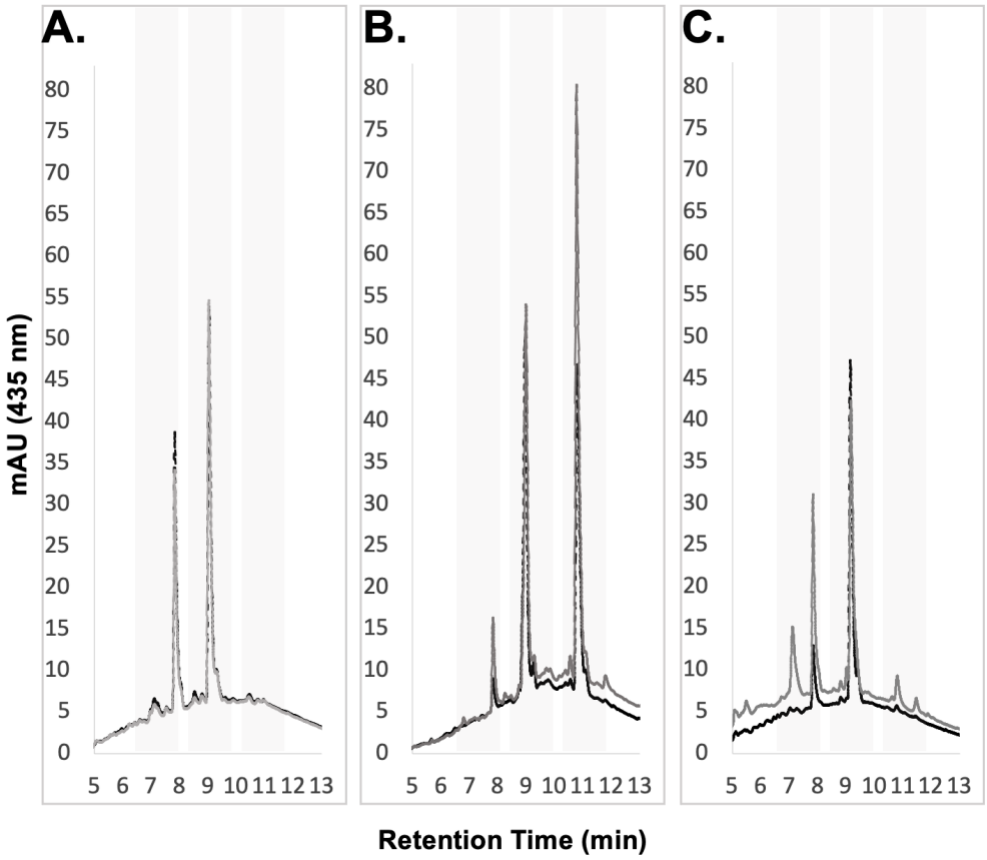
Supplementary Figure S9. Turnerbactin-like BGC phylogeny of *Serratia*. 50 turnerbactin-like BGCs were used to reconstruct this phylogeny using the conserved proteins present in all BGCs. Genomic context visualization, as well as in-deep functional annotation of each BGC, reveal two different catechol-type BGCs. Siderophore (C) BGC is present in all the *Serratia* (meta)genomes obtained from cycadivorous guts. Phylogenetic tree was constructed using a Bayesian method, employing a mixed substitution model over the course of 100,000 generations.



Supplementary Figure S10. Turnerbactin-like BGC phylogeny of *Stenotrophomonas*. 38 turnerbactin-like BGCs were used to reconstruct this phylogeny using the conserved proteins present in all BGCs. Genomic context visualization, as well as in-deep functional annotation of each BGC, reveal three *bona fide* catechol-type BGCs (D, E, and F), plus three siderophore-like BGCs, some of them present in *Stenotrophomonas* (meta)genomes obtained from cycadivorous guts. Phylogenetic tree was constructed using a Bayesian method, employing a mixed substitution model over the course of 100,000 generations.



Supplementary Figure S11. Turnerbactin-like BGC phylogeny of *Serratia-Pantoea*. 41 enterobactin-like BGCs from both *Serratia* and *Pantoea* genomes were used to reconstruct this phylogeny. Genomic context visualization, as well as in-deep functional annotation of each BGC, reveal one siderophore-like BGC present in some *Serratia* and *Pantoea* genomes plus a catechol-type BGC (G) present exclusively in *Serratia* (meta)genomes. Phylogenetic tree was constructed using a Bayesian method, employing a mixed substitution model over the course of 100,000 generations.



Supplementary Figure S12. Identification of siderophores produced by six bacterial strains isolated from cycadivorus insects through HPLC. HPLC analysis of **A.** Two *Serratia* strains: PF2-63 (dash line) and PF-27 (solid line), **B.** Two *Pantoea* strains: EA-12 (dash line) and EABMAA-21 (solid line), and **C.** Two *Stenotrophomonas:* PFBMAA-4 (dash line) and RS-48 (solid line) under siderophore-promoting conditions revealed signals at 435 nm associated with the production of these compounds. The indicated retention times (gray selection) were then collected and analyzed by MS-MS mass spectrometry.