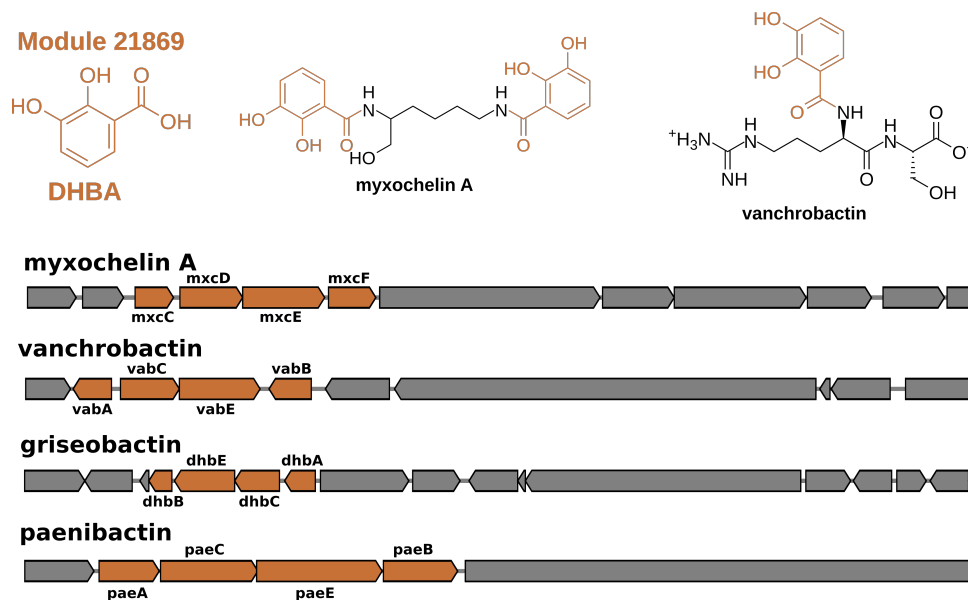
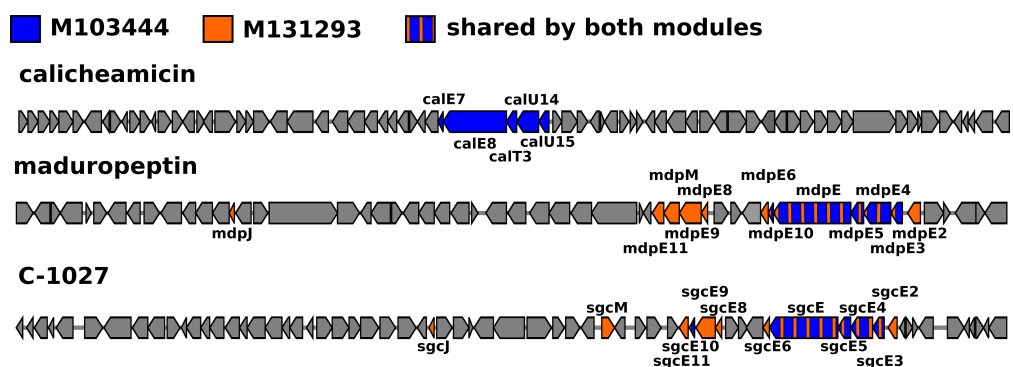


**Supplementary Figure 1: 4-methyl-3-hydroxyanthranilic acid (4-MHA).** The biosynthesis of 4-methyl-3-hydroxyanthranilic acid (4-MHA) is encoded by a sub-cluster of 3 genes present in at least 3 different BGCs: sibiromycin (*sibK*, *P*, *Q*)<sup>1</sup>, actinomycin (*acmF*, *G*, *H*)<sup>2</sup> and anthramycin (ABW71851.1, ABW71848.1 and ABW71847.1)<sup>3</sup>. As shown in Supplementary Figure 1, this sub-cluster corresponds to module M108999 (containing smCOG10870, smCOG11911 and smCOG12486; MIB score = 87.54, found in 11 BGCs). Furthermore, the module is present in the antimycin BGC. In the antimycin biosynthetic pathway, homologs of these same three genes are involved in the biosynthesis of 3-aminosalicylate<sup>4</sup>, which is structurally closely related to the final precursor of 4-MHA, 3-hydroxyanthranilic acid (3-HA). The methyltransferase that converts 3-HA to 4-MHA (which is not part of module M108999 because it is only present in a minority of the BGCs) is missing in the actinomycin pathway, which explains the additional structural difference. Interestingly, the shared genetic basis of the biosynthesis of these two nonproteinogenic amino acids had not been noted in literature thus far. Additionally, module M108999 contains seven uncharacterized BGCs from diverse organisms, including *Deinococcus*, *Streptomyces*, *Streptosporangium*, and *Nocardia* species. The 4-MHA-type sub-clusters in these BGCs are associated with a wide variety of core scaffold biosynthesis genes, including NRPSs, type I PKSs, type III PKSs and terpene cyclases. This suggests that biosynthetic pathways have evolved in which 4-MHA-like moieties are incorporated into a wide range of scaffolds. The detection of this cluster as statistically significant, despite the fact that the genes involved are not always contiguous, shows the power of the co-occurrence analysis included in the module detection algorithm. The 4-MHA moiety highlighted in the structures.



**Supplementary Figure 2: 2,3-dihydroxy-benzoic acid (DBHA).** Another well-known sub-cluster is represented by a group of 4 genes encoding the biosynthesis of 2,3-dihydroxy-benzoic acid (DHBA)<sup>5</sup>. This sub-cluster has been described in several characterised BGCs: myxochelin A (*mxC*, *D*, *E*, *F*)<sup>6</sup>, vanchrobactin (*vabA*, *C*, *E*, *B*)<sup>7</sup>, paenibactin (*paeA*, *C*, *E*, *B*)<sup>8</sup>, griseobactin (*dhbA*, *C*, *E*, *B*)<sup>9</sup>, enterobactin (*entA*, *C*, *E*, *B*)<sup>10</sup> and vibriobactin (*vibA*, *C*, *E*, *B*)<sup>11</sup>. As shown in Supplementary Figure 2, module M21869 perfectly covers this sub-cluster for 4 of the above mentioned clusters (MIB score = 77.58, number of BGCs covered = 299). Whilst the vibriobactin cluster is not present in our initial dataset, module M21869 does not target the BGC encoding enterobactin. This BGC is targeted instead by module M21893 (MIB score = 58.94, BGCs covered = 306), comprising a subset of module M21869, lacking the smCOG covering the *entA* gene, as *entA* is assigned to a different smCOG (smCOG11931, often annotated as amino acid adenylation domain) by our automated annotation algorithm. This case illustrates two main points: (1) the use of variable significance thresholds allows the detection of partial matches to biosynthetic modules, and (2) the module detection algorithm is robust towards minor misannotations in individual gene clusters, which will be unavoidable for any large-scale genomic analysis. The DHBA moiety highlighted in the structures. The structures for griseobactin and paenibactin are not available in ChemSpider nor PubChem.



**Supplementary Figure 3: Enediyne.** The biosynthetic logic of enediyne specialised metabolites remains largely enigmatic when compared to other polyketides<sup>12</sup>. Irrespectively, our analysis identified one module, M103444, to completely cover the group of genes responsible for the core biosynthesis of both 9- and 10-membered enediyne<sup>13</sup> indiscriminately. The existence of a sub-cluster of core genes conserved between 9 and 10-membered enediyne BGCs is well reported in literature, and this gene cassette is routinely used as a probe to mine sequence data for new and exotic enediyne-specialised metabolites<sup>14, 15</sup>. The factors discriminating between the biosynthesis of 9- and 10-membered enediyne rings remain unknown however, but are thought to occur beyond the “core” biosynthetic genes within module M103444. Genome neighbourhood network analysis of 10 characterised enediyne BGCs highlighted 9 genes to associate specifically with biosynthesis of 9-membered enediyne (D2, E2, E8, E9, E11, F, J, L and M), and 4 genes to associate with 10-membered enediyne ring biosynthesis (R3, S6, T5 and U20)<sup>14</sup>, none of which are covered by module M103444. Interestingly, as shown in Figure 3, module M103444 is encompassed within a larger module, M131293, which appears to be specific to 9-membered enediyne and in addition to module M103444, comprise 6 of the 9 9-member specific genes (E2, E8, E9, E11, J and M). The genes covered by module M03444 (blue), module M131293 (orange) or both (blue-and-orange stripes) are highlighted.

## Supplementary Note 1 - Ectoine and $\beta$ -carotene

Module M107196 (MIB score = 83.44) contains 3 smCOGs (smCOG1005, smCOG10036 and smCOG10195) that cover genes involved in the biosynthesis of  $\beta$ -carotene from geranylgeranyl diphosphate (GGPP)<sup>16,17</sup>. This module is found in 139 BGCs, 8 of which have been experimentally characterised and reported using the MIBiG standard<sup>18</sup>. Despite the wide distribution of this module among the predicted BGCs present in our dataset, it is always found in very similar genetic contexts; therefore, it shows a very low Shannon entropy value (0.47). In fact 94% of the BGCs are annotated as terpenes, and all the others are annotated as hybrid clusters also including terpenes (e.g. terpene-bacteriocin). All 8 chemically characterised BGCs produce highly similar carotenoids where the “sub-cluster” is responsible for biosynthesis of the core specialised metabolite, opposed to a discrete chemical moiety. A similar situation is found with module M112779 (MIB score = 82.40), which encodes the enzymatic machinery necessary for ectoine biosynthesis<sup>19</sup>. This small module containing 3 smCOGs (smCOG10060, smCOG10107 and smCOG10163, corresponding to *ectB*, *ectC* and *ectA*), is prevalent, being found in 235 BGCs, and has a very low Shannon entropy value (0.37). Such low Shannon entropy indicates that these modules exist as discrete BGCs encoding ectoine and carotenoids, rather than a module within a larger parent BGC synthesising a complex hybrid specialised metabolite, e.g. simocyclinone, calicheamicin or coumermycin.

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