Supporting Information for:

The Natural Product Domain Seeker version 2 (NaPDoS2) webtool relates ketosynthase phylogeny to biosynthetic function

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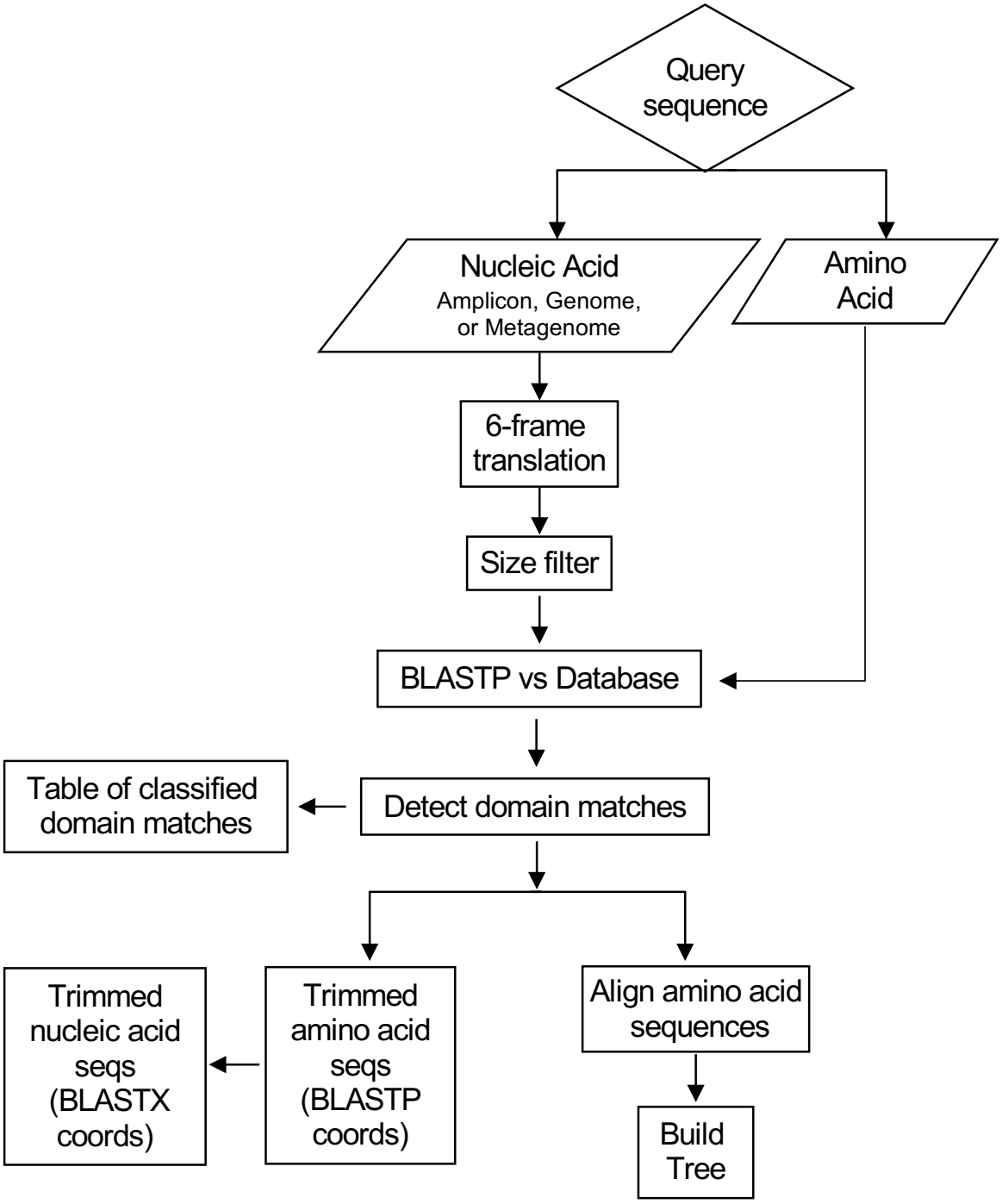


Figure S1. NaPDoS2 bioinformatic pipeline. Translated nucleotide and protein query sequences are compared to an internal database of KS and C domains using BLASTP. Detailed descriptions of individual steps can be found in the GitHub repository site: https://github.com/spodell/NaPDoS2_website.

Figure S2.

A.

B.

C.

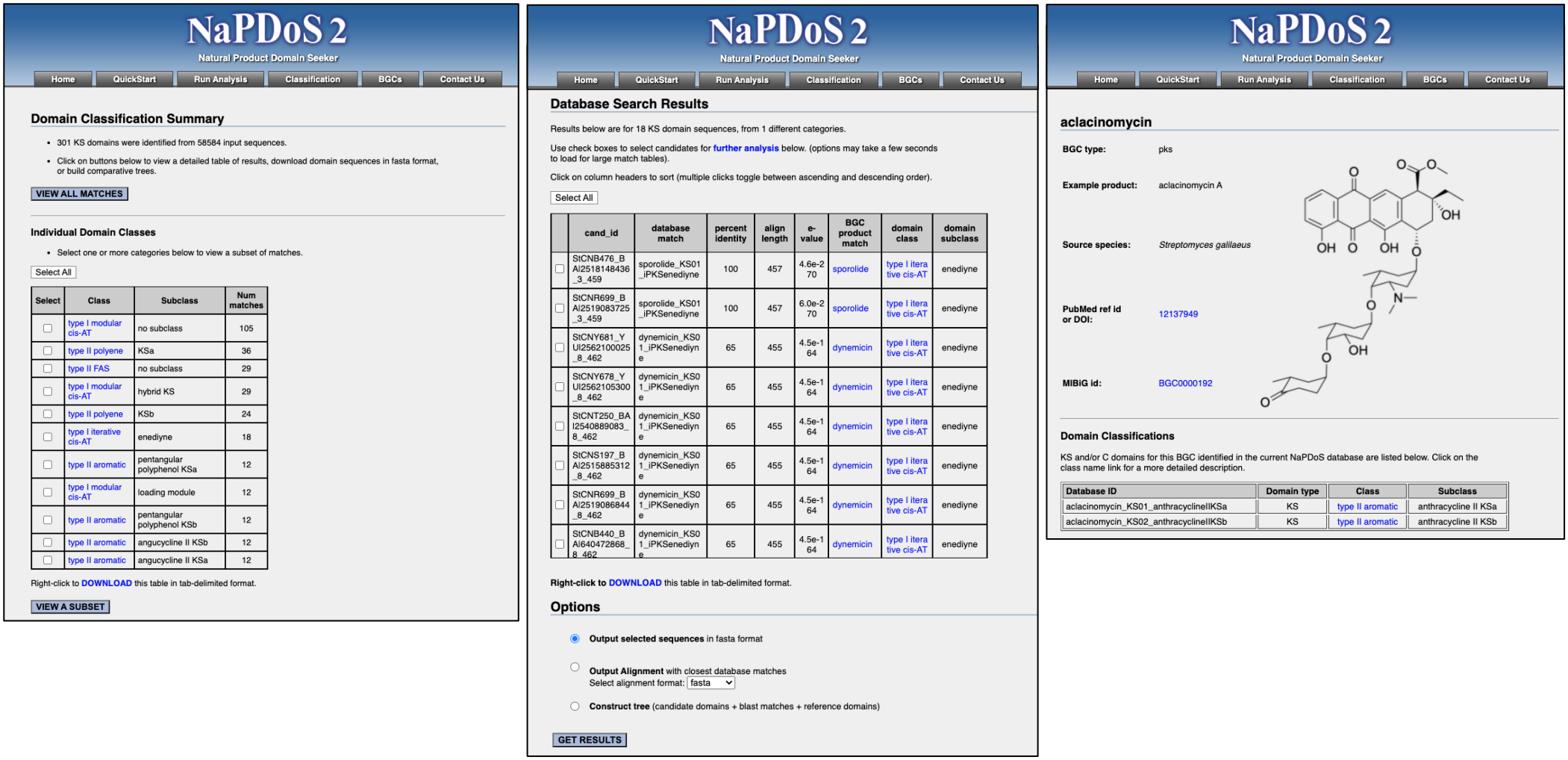
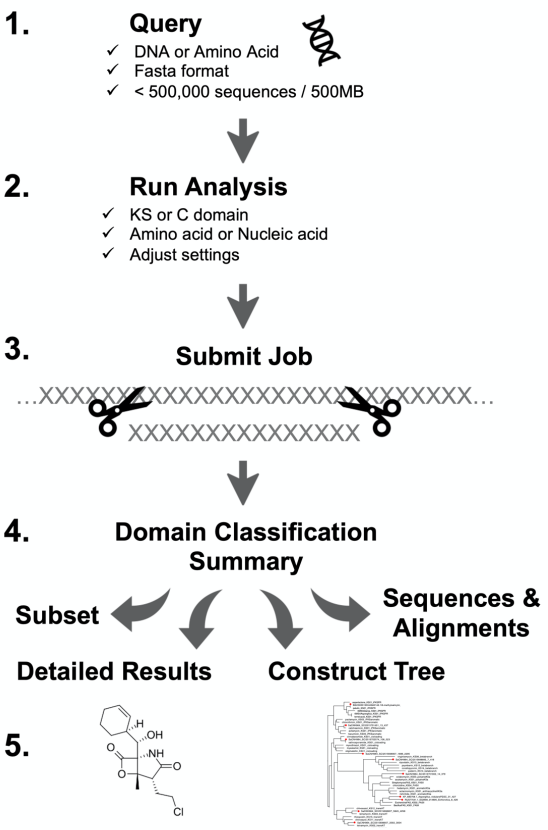


Figure S2. **Featured webtool updates**. A). Domain classification summary page. In this example, 301 KS domains were detected in twelve bacterial genomes (58,584 protein sequences). The number of domains detected in each class and subclass is indicated. B). Database search results page. Expanded view of the 18 type I iterative *cis*-AT enediyne domains from the search shown in (A). C). BGC page. Clicking on the BGC product match hyperlink from the Database Search Results in panel (B) provides the compound structure and details about the associated BGC and all corresponding KS classifications (C domains to be updated in a later release).

Figure S3.

A.


1.
Query
✓ DNA or Amino Acid
✓ Fasta format
✓ < 500,000 sequences / 500MB
2.
Run Analysis
✓ KS or C domain
✓ Amino acid or Nucleic acid
✓ Adjust settings
3.
Submit Job
4.
Domain Classification Summary
Subset
Sequences & Alignments
Detailed Results
Construct Tree
5.

B.

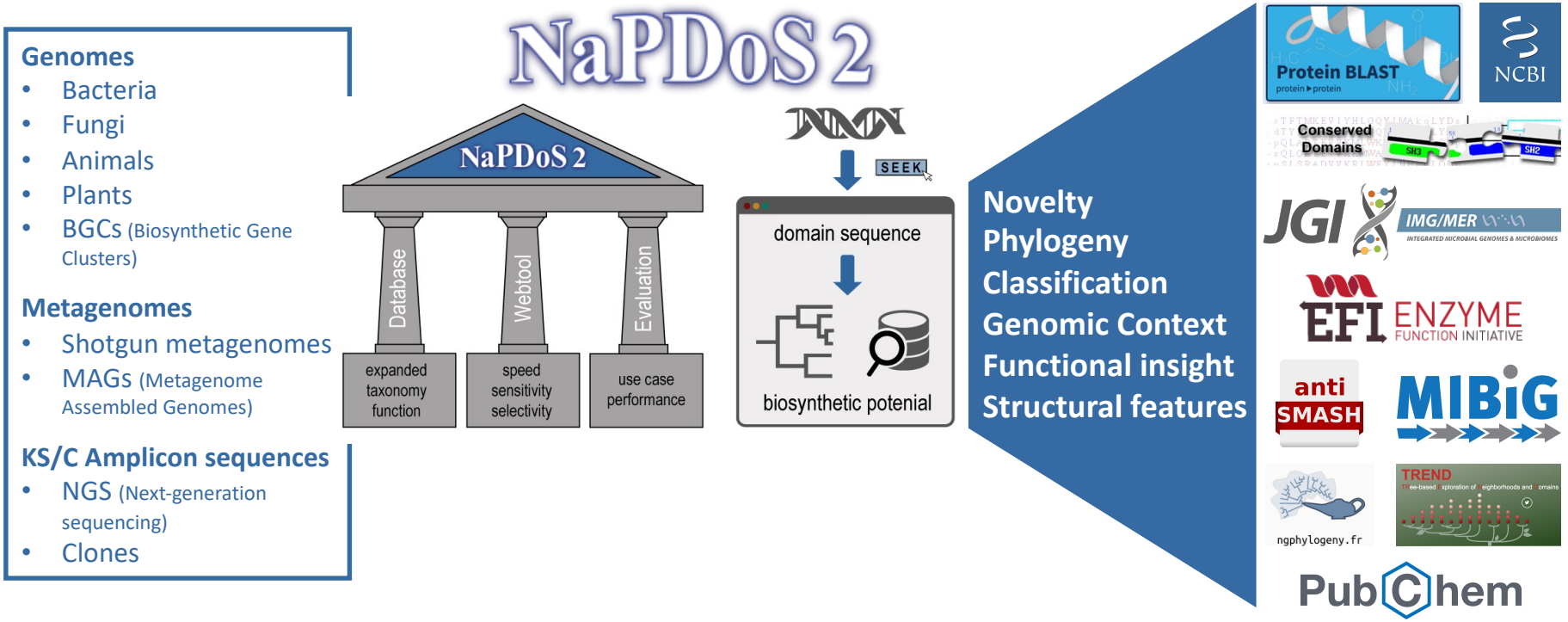

Genomes
• Bacteria
• Fungi
• Animals
• Plants
• BGCs (Biosynthetic Gene Clusters)
Metagenomes
• Shotgun metagenomes
• MAGs (Metagenome Assembled Genomes)
KS/C Amplicon sequences
• NGS (Next-generation sequencing)
• Clones
NaPDoS 2
NaPDoS 2
Database
Webtool
Evaluation
expanded taxonomy function
speed sensitivity selectivity
use case performance
SEEK
domain sequence
biosynthetic potenial
Novelty
Phylogeny
Classification
Genomic Context
Functional insight
Structural features
Protein BLAST
protein ▸ protein
NCBI
Conserved Domains
SH3
SH2
JGI
IMG/MER
INTEGRATED MICROBIAL GENOMES & MICROBIOMES
EFI ENZYME FUNCTION INITIATIVE
anti SMASH
MIBiG
ngphylogeny.fr
TREND
PubChem

Figure S3. NaPDoS2 workflow and analysis roadmap. A) The NaPDoS2 user workflow consists of submitting a query sequence, selecting the type of analysis to run, submitting the job, and deciding what output to view or analyses to complete. B) A roadmap for the use of NaPDos2 starts with genomic, metagenomic, or KS/C domain amplicon sequences derived from a variety of sources. The expanded database and webtool improvements provide important analytical upgrades while extensive use testing demonstrates the applications of NaPDoS2 to assess biosynthetic potential by detecting and classifying KS and C domain sequences. NaPDoS2 output can be further analyzed using a variety of tools to assess novelty, phylogeny, classification, genomic context, function, and small molecule structural features. Detailed, step-by-step tutorial examples can be found in the downloadable “Documentation” PDF linked on the NaPDoS2 webpage.

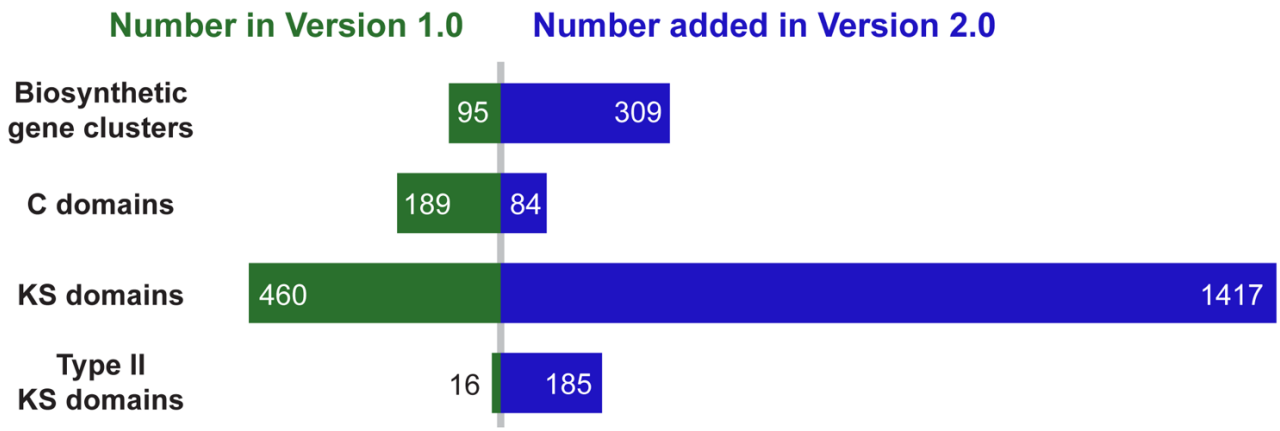
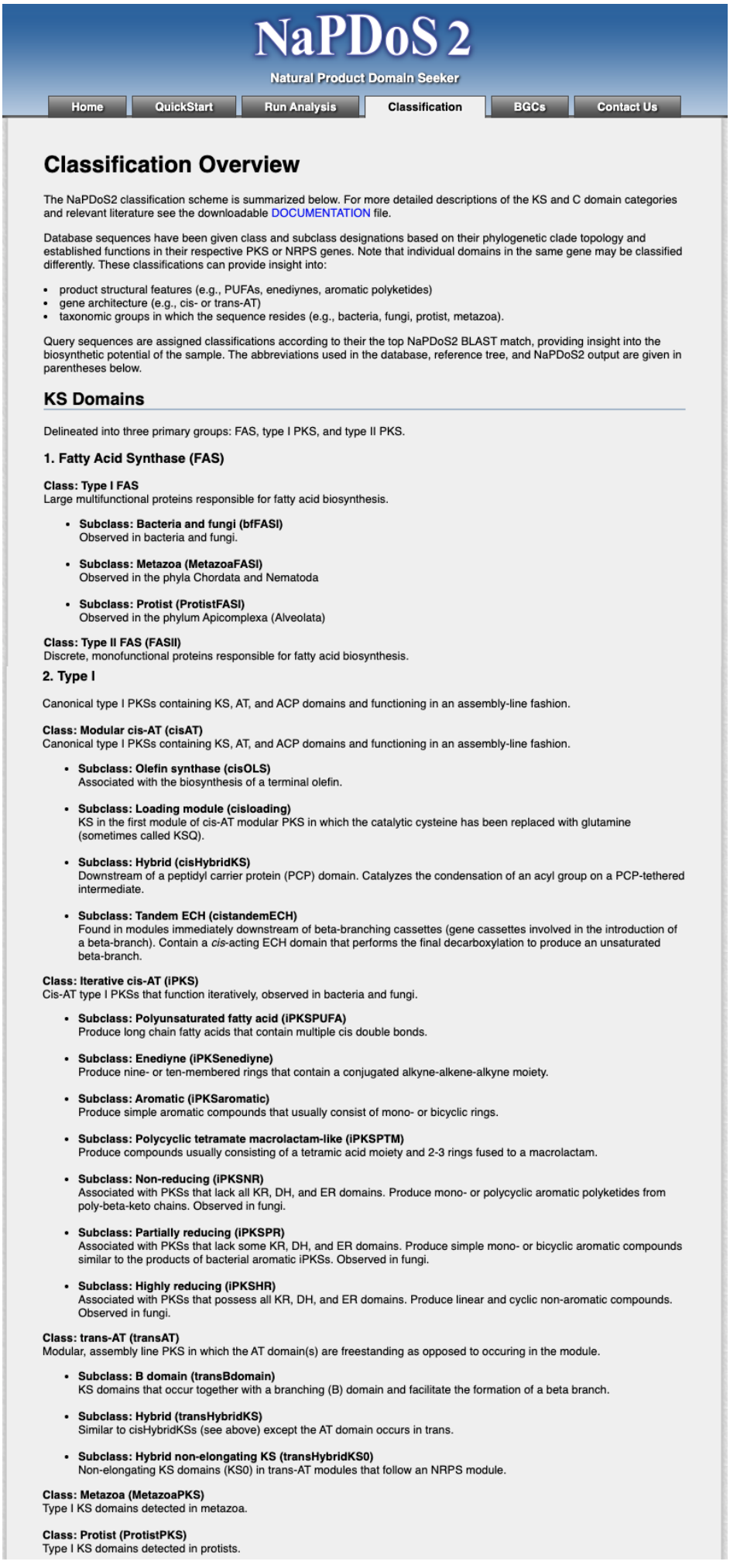


Figure S4. Comparison of the expanded NaPDoS2 database with the original NaPDoS version 1 release[1]. The NaPDoS2 reference database contains 273 C domains and 1,877 KS domains from a total of 404 BGCs.

Figure S5.



NaPDoS 2

Natural Product Domain Seeker

Home | QuickStart | Run Analysis | Classification | BGCs | Contact Us

Classification Overview

The NaPDoS2 classification scheme is summarized below. For more detailed descriptions of the KS and C domain categories and relevant literature see the downloadable DOCUMENTATION file.

Database sequences have been given class and subclass designations based on their phylogenetic clade topology and established functions in their respective PKS or NRPS genes. Note that individual domains in the same gene may be classified differently. These classifications can provide insight into:

- product structural features (e.g., PUFAs, enediynes, aromatic polyketides)
- gene architecture (e.g., cis- or trans-AT)
- taxonomic groups in which the sequence resides (e.g., bacteria, fungi, protist, metazoa).

Query sequences are assigned classifications according to their the top NaPDoS2 BLAST match, providing insight into the biosynthetic potential of the sample. The abbreviations used in the database, reference tree, and NaPDoS2 output are given in parentheses below.

KS Domains

Delineated into three primary groups: FAS, type I PKS, and type II PKS.

1. Fatty Acid Synthase (FAS)

Class: Type I FAS
Large multifunctional proteins responsible for fatty acid biosynthesis.

- **Subclass: Bacteria and fungi (bfFASI)**
  Observed in bacteria and fungi.
- **Subclass: Metazoa (MetazoaFASI)**
  Observed in the phyla Chordata and Nematoda
- **Subclass: Protist (ProtistFASI)**
  Observed in the phylum Apicomplexa (Alveolata)

Class: Type II FAS (FASII)
Discrete, monofunctional proteins responsible for fatty acid biosynthesis.

2. Type I

Canonical type I PKSs containing KS, AT, and ACP domains and functioning in an assembly-line fashion.

Class: Modular cis-AT (cisAT)
Canonical type I PKSs containing KS, AT, and ACP domains and functioning in an assembly-line fashion.

- **Subclass: Olefin synthase (cisOLS)**
  Associated with the biosynthesis of a terminal olefin.
- **Subclass: Loading module (cisloading)**
  KS in the first module of cis-AT modular PKS in which the catalytic cysteine has been replaced with glutamine (sometimes called KSQ).
- **Subclass: Hybrid (cisHybridKS)**
  Downstream of a peptidyl carrier protein (PCP) domain. Catalyzes the condensation of an acyl group on a PCP-tethered intermediate.
- **Subclass: Tandem ECH (cistandemECH)**
  Found in modules immediately downstream of beta-branching cassettes (gene cassettes involved in the introduction of a beta-branch). Contain a *cis*-acting ECH domain that performs the final decarboxylation to produce an unsaturated beta-branch.

Class: Iterative cis-AT (iPKS)
Cis-AT type I PKSs that function iteratively, observed in bacteria and fungi.

- **Subclass: Polyunsaturated fatty acid (iPKSPUFA)**
  Produce long chain fatty acids that contain multiple cis double bonds.
- **Subclass: Enediyne (iPKSenediyne)**
  Produce nine- or ten-membered rings that contain a conjugated alkyne-alkene-alkyne moiety.
- **Subclass: Aromatic (iPKSaromatic)**
  Produce simple aromatic compounds that usually consist of mono- or bicyclic rings.
- **Subclass: Polycyclic tetramate macrolactam-like (iPKSPTM)**
  Produce compounds usually consisting of a tetramic acid moiety and 2-3 rings fused to a macrolactam.
- **Subclass: Non-reducing (iPKSNR)**
  Associated with PKSs that lack all KR, DH, and ER domains. Produce mono- or polycyclic aromatic polyketides from poly-beta-keto chains. Observed in fungi.
- **Subclass: Partially reducing (iPKSPR)**
  Associated with PKSs that lack some KR, DH, and ER domains. Produce simple mono- or bicyclic aromatic compounds similar to the products of bacterial aromatic iPKSs. Observed in fungi.
- **Subclass: Highly reducing (iPKSHR)**
  Associated with PKSs that possess all KR, DH, and ER domains. Produce linear and cyclic non-aromatic compounds. Observed in fungi.

Class: trans-AT (transAT)
Modular, assembly line PKS in which the AT domain(s) are freestanding as opposed to occuring in the module.

- **Subclass: B domain (transBdomain)**
  KS domains that occur together with a branching (B) domain and facilitate the formation of a beta branch.
- **Subclass: Hybrid (transHybridKS)**
  Similar to cisHybridKSs (see above) except the AT domain occurs in trans.
- **Subclass: Hybrid non-elongating KS (transHybridKS0)**
  Non-elongating KS domains (KS0) in trans-AT modules that follow an NRPS module.

Class: Metazoa (MetazoaPKS)
Type I KS domains detected in metazoa.

Class: Protist (ProtistPKS)
Type I KS domains detected in protists.

Figure S5.

3. Type II

Discrete, monofunctional proteins.

Class: Aromatic (aromaticKSa or aromaticKSb)
Heterodimers that consist of alpha and beta subunits and produce polycyclic aromatic compounds through the iterative decarboxylative condensation of malonyl-CoA extender units onto an acyl starting unit.

- **Subclass: angucycline-derived I (angucyclineIKSa or angucyclineIKSb)**
  Compounds contain or were derived from an angular tetracyclic structure comprising a benzanthracene moiety. Most frequently initiated with acetyl-CoA starting unit.
- **Subclass: angucycline-derived II (angucyclineIIKSa or angucyclineIIKSb)**
  Distinguished from angucycline-derived I by initiation with a methylmalonyl-CoA starting unit.
- **Subclass: anthracycline-derived I (anthracyclineIKSa or anthracyclineIKSb)**
  Compounds possess a linear tetracyclic core derived from 7,8,9,10-tetrahydro-5,12-naphtacenoquinones. Initiated with acetyl-CoA starting unit.
- **Subclass: anthracycline-derived II (anthracyclineIIKSa or anthracyclineIIKSb)**
  Distinguished from anthracycline-derived I by initiation with methylmalonyl-CoA starting unit.
- **Subclass: isochromanequinone-derived (isochromanequinoneKSa or isochromanequinoneKSb).**
  Compounds with a linear tricyclic core structure containing isochromane and quinone moieties often forming dimers.
- **Subclass: pentangular polyphenol-derived (pentangularpolyphenolKSa or pentangularpolyphenolKSb)**
  Produce long-chain polyphenols that form angular polycyclic core structures.
- **Subclass: tetracenomycin-derived (tetracenomycinKSa or tetracenomycinKSb)**
  Produce linear tetracyclic decaketide core structures resulting from nine elongations of an acetyl-CoA starting unit.
- **Subclass: tetracycline-derived (tetracyclineKSa) or (tetracyclineKSb)**
  Produce compounds with a tetracyclic ring structure characterized by a carboxamido moiety resulting from a malonamyl-CoA starting unit.
- **Subclass: spore pigment (sporepigmentKSa or sporepigmentKSb)**
  Associated with the biosynthesis of streptomycete spore pigments (e.g. whiE in S. coelicolor) although compounds are not well characterized.

Class: Beta-branching cassettes (betabranch)
KS domains associated with HMGS cassettes that introduce a beta-branch to a beta-keto group. These stand alone KSs lack the active site cysteine required for condensation and function to decarboxylate ACP-bound malonyl as an early step in beta branch formation

Class: Polyenes (polyeneKSa or polyeneKSb)
Iteratively acting KSs that produce reduced, linear polyenes rather than polycyclic aromatic compounds.

Class: Aryl polyenes (arylpolyeneKSa or arylpolyeneKSb)
Iteratively acting PKSs that produce polyene chains with an aryl moiety that is often substituted.

Class: Non-iterative (noniterative)
Discrete monofunctional proteins that function as an assembly line to produce compounds such as pamamycin and nonactin.

C Domains

Class: Starter (starter)
Typically, the first module of a NRPS usually does not contain a C domain. But, when present, these starter C domains acylate the first amino acid with a fatty acid, polyketide, or other molecule.

Class: LCL (LCL)
Catalyzes the formation of a peptide bond between two L-amino acids.

Class: DCL (DCL)
Catalyzes the formation of a peptide bond between an L-amino acid and a growing peptide ending with a D-amino acid.

Class: Cyclization (cyclization)
Catalyzes both peptide bond formation and the subsequent cyclization of cysteine, serine or threonine residues.

Class: Epimerization (epimerization)
Changes the chirality of the last amino acid in the chain from L to D.

Class: Dual (dual)
Catalyzes both condensation and epimerization reactions.

Class: Modified amino acid (modifiedAA)
Modifies the incorporated amino acid: for example the dehydration of serine to dehydroalanine.

Class: Hybrid (hybridC)
Occur in PKS-NRPS BGCs. The condensation domain that occurs immediately downstream of a PKS module; condenses an amino acid to a growing polyketide.

Class: Condensation (condensation)
Condensation domains with no known specialized functionality.

Figure S5. NaPDoS2 classification overview. Class and subclass descriptions as described on the NaPDoS2 website. Additional details and relevant references can be found in the downloadable “Documentation” PDF linked at the top of the webpage.

Figure S6.

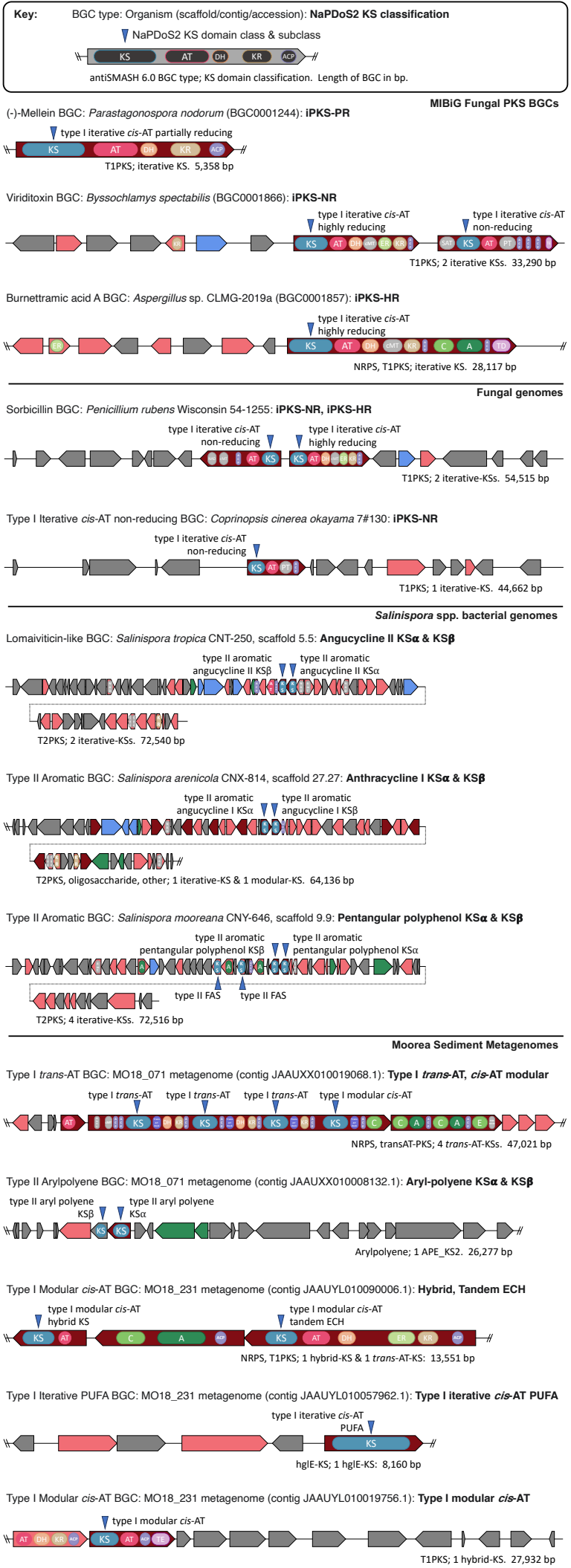

Key: BGC type: Organism (scaffold/contig/accession): NaPDoS2 KS classification
NaPDoS2 KS domain class & subclass
KS AT DH KR ACP
antiSMASH 6.0 BGC type; KS domain classification. Length of BGC in bp.
MIBiG Fungal PKS BGCs
(-)-Mellein BGC: Parastagonospora nodorum (BGC0001244): iPKS-PR
type I iterative cis-AT partially reducing
T1PKS; iterative KS. 5,358 bp
Viriditoxin BGC: Byssochlamys spectabilis (BGC0001866): iPKS-NR
type I iterative cis-AT highly reducing
type I iterative cis-AT non-reducing
T1PKS; 2 iterative KSs. 33,290 bp
Burnettramic acid A BGC: Aspergillus sp. CLMG-2019a (BGC0001857): iPKS-HR
type I iterative cis-AT highly reducing
NRPS, T1PKS; iterative KS. 28,117 bp
Fungal genomes
Sorbicillin BGC: Penicillium rubens Wisconsin 54-1255: iPKS-NR, iPKS-HR
type I iterative cis-AT non-reducing
type I iterative cis-AT highly reducing
T1PKS; 2 iterative-KSs. 54,515 bp
Type I Iterative cis-AT non-reducing BGC: Coprinopsis cinerea okayama 7#130: iPKS-NR
type I iterative cis-AT non-reducing
T1PKS; 1 iterative-KS. 44,662 bp
Salinispora spp. bacterial genomes
Lomaiviticin-like BGC: Salinispora tropica CNT-250, scaffold 5.5: Angucycline II KSα & KSβ
type II aromatic angucycline II KSβ
type II aromatic angucycline II KSα
T2PKS; 2 iterative-KSs. 72,540 bp
Type II Aromatic BGC: Salinispora arenicola CNX-814, scaffold 27.27: Anthracycline I KSα & KSβ
type II aromatic angucycline I KSα
type II aromatic angucycline I KSβ
T2PKS, oligosaccharide, other; 1 iterative-KS & 1 modular-KS. 64,136 bp
Type II Aromatic BGC: Salinispora mooreana CNY-646, scaffold 9.9: Pentangular polyphenol KSα & KSβ
type II aromatic pentangular polyphenol KSβ
type II aromatic pentangular polyphenol KSα
type II FAS
type II FAS
T2PKS; 4 iterative-KSs. 72,516 bp
Moorea Sediment Metagenomes
Type I trans-AT BGC: MO18_071 metagenome (contig JAAUXX010019068.1): Type I trans-AT, cis-AT modular
type I trans-AT
type I trans-AT
type I trans-AT
type I modular cis-AT
NRPS, transAT-PKS; 4 trans-AT-KSs. 47,021 bp
Type II Arylpolyene BGC: MO18_071 metagenome (contig JAAUXX010008132.1): Aryl-polyene KSα & KSβ
type II aryl polyene KSβ
type II aryl polyene KSα
Arylpolyene; 1 APE_KS2. 26,277 bp
Type I Modular cis-AT BGC: MO18_231 metagenome (contig JAAUYL010090006.1): Hybrid, Tandem ECH
type I modular cis-AT hybrid KS
type I modular cis-AT tandem ECH
NRPS, T1PKS; 1 hybrid-KS & 1 trans-AT-KS: 13,551 bp
Type I Iterative PUFA BGC: MO18_231 metagenome (contig JAAUYL010057962.1): Type I iterative cis-AT PUFA
type I iterative cis-AT PUFA
hglE-KS; 1 hglE-KS: 8,160 bp
Type I Modular cis-AT BGC: MO18_231 metagenome (contig JAAUYL010019756.1): Type I modular cis-AT
type I modular cis-AT
T1PKS; 1 hybrid-KS. 27,932 bp

Figure S6. **Verification of NaPDoS2 KS domain classifications.** Biosynthetic gene cluster context of select KSs detected in the application use case analyses. For each KS, the associated scaffold or contig was extracted and run through antiSMASH 6.0[2] (https://antismash.secondarymetabolites.org/), transATor[3] (https://transator.ethz.ch/), and the "PKS/NRPS Analysis Web-site"[4] (http://nrps.igs.umaryland.edu/). The BGCs were drawn and colored as determined by antiSMASH 6.0 (maroon, core biosynthetic gene; pink, additional biosynthetic gene; blue, transport-related genes; green, regulatory genes, grey, other genes). Domain position and function were drawn and colored according to antiSMASH 6.0[2], transATor[3], and "PKS/NRPS Analysis Web-site"[4] (blue, KS ketosynthase; pink, AT acyl transferase; sand, KR ketoreductase; pale purple ACP Phosphopantetheine acyl carrier protein, ACPS holo-ACP synthase; orange, DH dehydratase; light grey, cMT carbon methyltransferase, FkbH domain, NAD Male sterility protein, AmT aminotransferase, Cyc cyclase, GNAT GNAT domain, TIGR01720 NRPS domain of unknown function; light pink, TE thioesterase, TD Terminal reductase domain; pale green ER enoyl reductase; dark blue, *trans*-AT docking *trans*-acyltransferase docking domain; light green, C condensation domain of NRPS, E epimerization domain; dark green, A adenylation domain). Blue arrows point to KS hits that NaPDoS2 detected and classified in the BGC context; arrows are labeled with the NaPDoS2 KS domain classification. The antiSMASH 6.0[2] BGC type and KS domain classification, followed by the length of the entire BGC (in base pairs) is listed below each BGC, as indicated in the key.

We strategically chose diverse use case analyses for ground-truthing the contextual genomic evidence for KS domain-based classification. NaPDoS2 correctly classified KSs associated with partially reducing ((-)-Mellein), non-reducing (viriditoxin), and highly reducing (burnettramic acid A) fungal BGCs from MIBiG 2.0[5], as confirmed by literature reports and antiSMASH 6.0[2] output. Next, NaPDoS2 correctly classified the KSs in the sorbicillin BGC from *Penicillium rubens* fungal genome, which contains both non-reducing and highly reducing KS domains. NaPDoS2 also identified a type I iterative *cis*-AT non-reducing KS in an orphan BGC in the *Coprinopsis cinerea okayama* basidiomycete genome. Next, NaPDoS2 also correctly identified the KS domains in the lomaiviticin BGC as type II aromatic angucycline II, and detected the KSs associated with two *Salinispora* orphan BGCs as type II aromatic anthracycline I and pentangular polyphenol. Finally, NaPDoS2 correctly classified *trans*-AT, *cis*-AT, aryl-polyene, hybrid, trandem ECH, modular, and PUFA KS domains from metagenomic assemblies based on their respective BGC context. In many cases, the NaPDoS2 classification was more specific than antiSMASH 6.0[2] BGC domain predictions.

Figure S7.

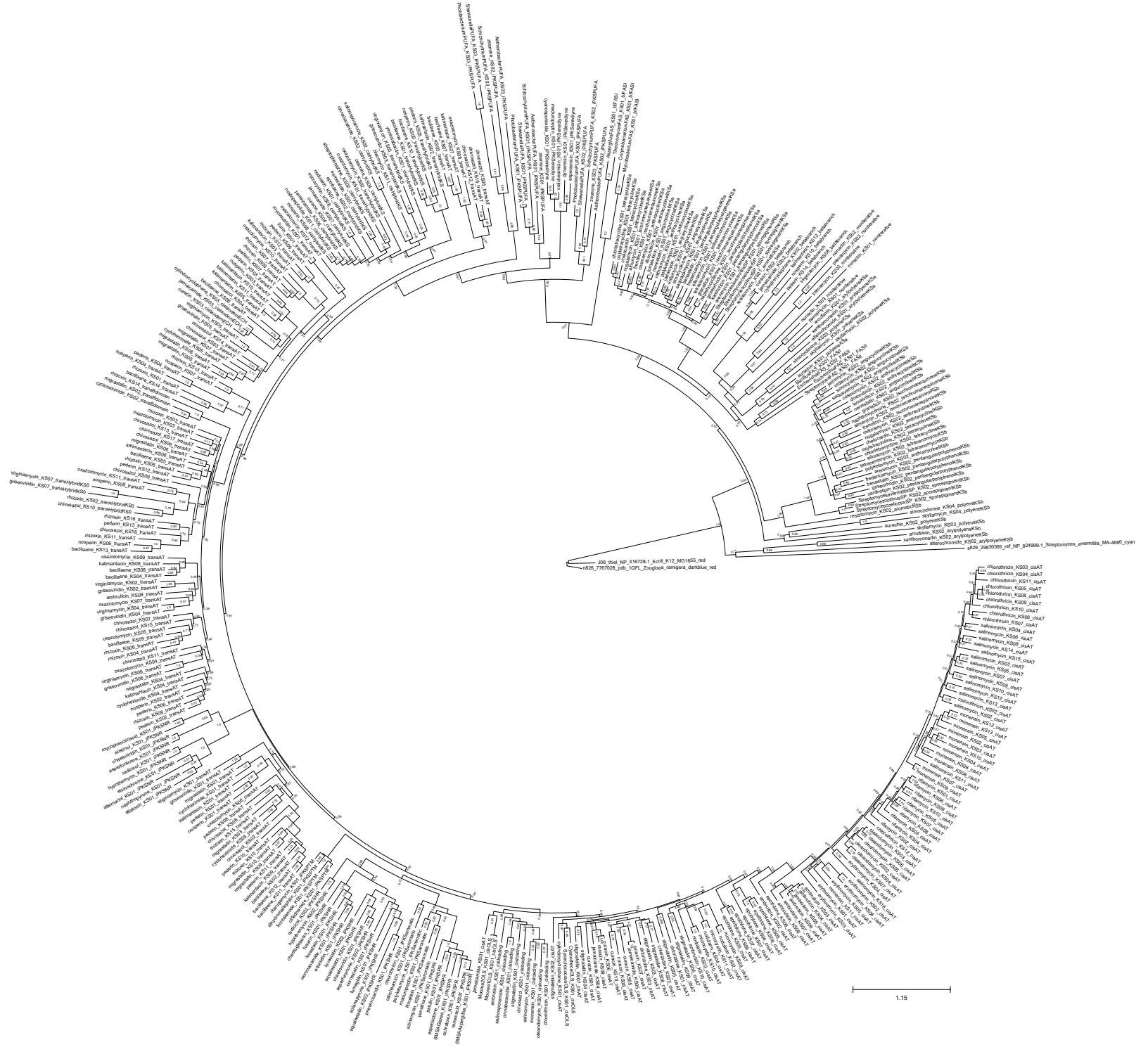

J08_thiol_NP_416728-1_Ecoli_K12_MG1655_red
n826_7767028_pdb_1QFL_Zoogloea_ramigera_darkblue_red
s829_29836365_ref_NP_824999-1_Streptomyces_avermitilis_MA-4680_cyan
1.15

Figure S7. **Maximum likelihood KS phylogeny.** Tree includes 414 KS sequences and 3 thiolase sequences as outgroups. The full name of each sequence is listed on the branch tips, which can help link a query match to a specific location in the tree. Bootstrap support is listed for each node. Thiolases from *Escherichia coli* (NP_416728.1) and *Zoogloea ramigera* (1QFL_A) and a SCP-x thiolase from *Streptomyces avermitilis* (NP_824999.1) were used as outgroups.

Figure S8.

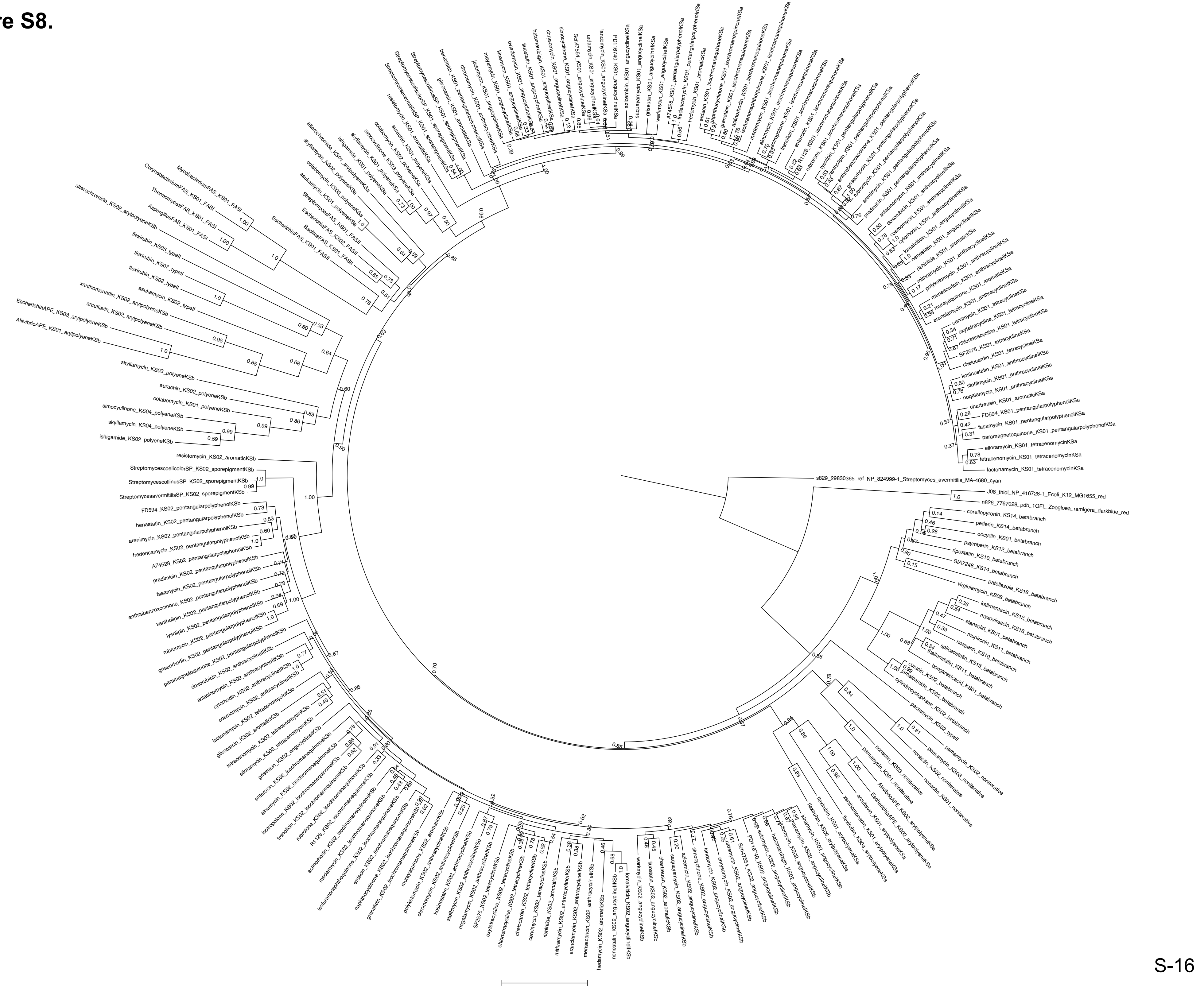


Figure S8. Maximum likelihood type II KS phylogeny. Tree includes 201 type II KS sequences, 8 FAS sequences and 3 thiolase sequences as outgroups. The full name of each sequence is listed on the branch tips, which can help link a query match to a specific location in the tree. Bootstrap support is listed for each node. Thiolases from *Escherichia coli* (NP_416728.1) and *Zoogloea ramigera* (1QFL_A) and a SCP-x thiolase from *Streptomyces avermitilis* (NP_824999.1) were used as outgroups.

Figure S9.

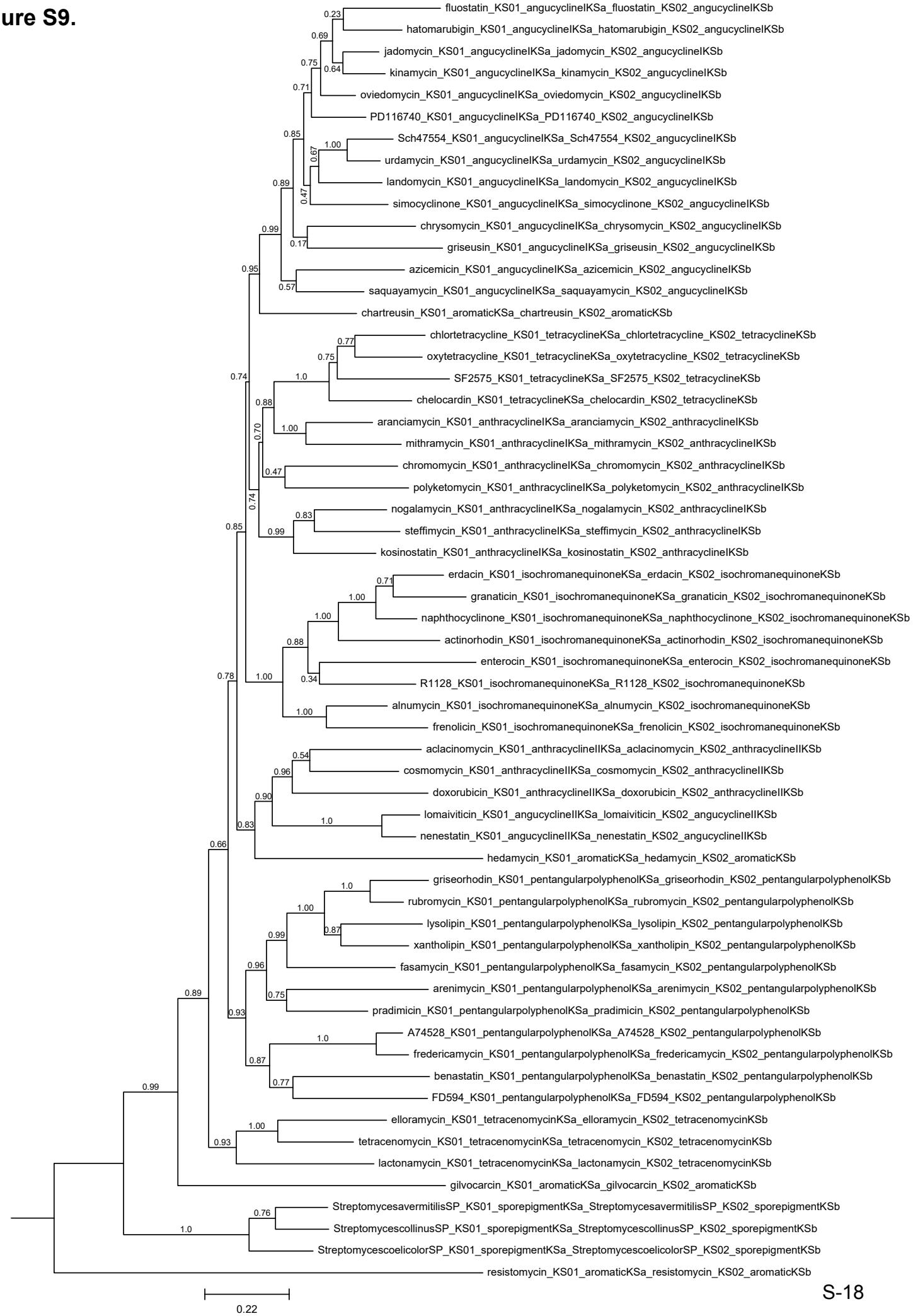
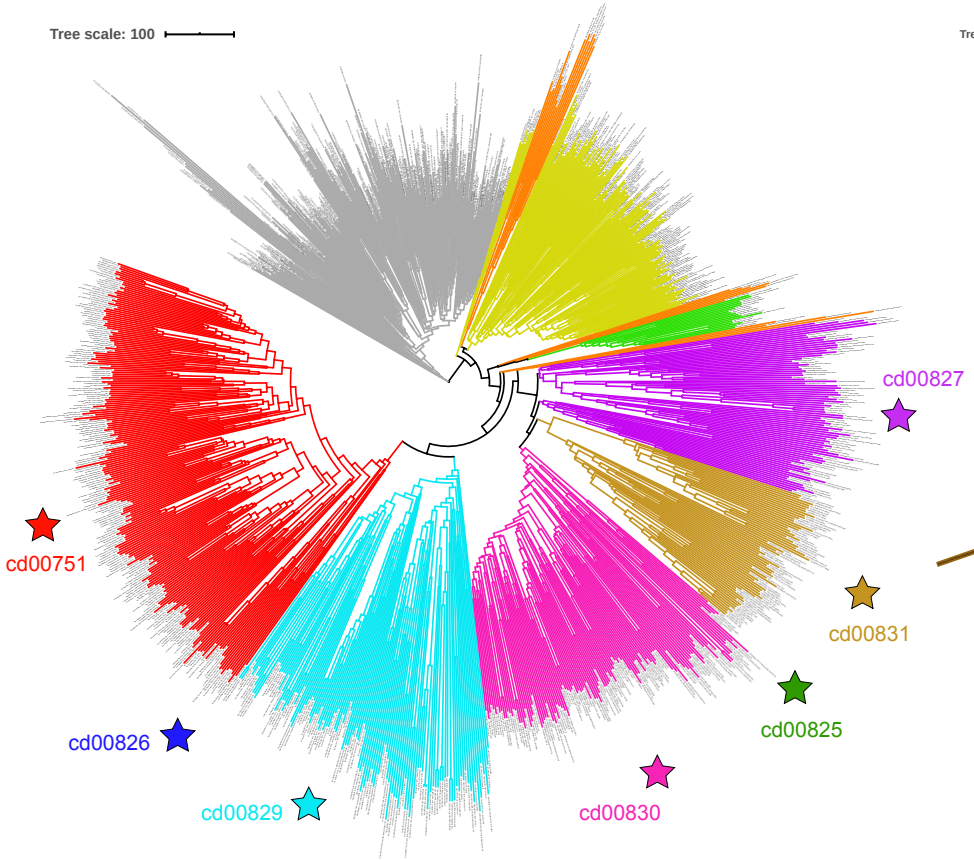

fluostatin_KS01_angucyclineIKSa_fluostatin_KS02_angucyclineIKSb
hatomarubigin_KS01_angucyclineIKSa_hatomarubigin_KS02_angucyclineIKSb
jadomycin_KS01_angucyclineIKSa_jadomycin_KS02_angucyclineIKSb
kinamycin_KS01_angucyclineIKSa_kinamycin_KS02_angucyclineIKSb
oviedomycin_KS01_angucyclineIKSa_oviedomycin_KS02_angucyclineIKSb
PD116740_KS01_angucyclineIKSa_PD116740_KS02_angucyclineIKSb
Sch47554_KS01_angucyclineIKSa_Sch47554_KS02_angucyclineIKSb
urdamycin_KS01_angucyclineIKSa_urdamycin_KS02_angucyclineIKSb
landomycin_KS01_angucyclineIKSa_landomycin_KS02_angucyclineIKSb
simocyclinone_KS01_angucyclineIKSa_simocyclinone_KS02_angucyclineIKSb
chrysomycin_KS01_angucyclineIKSa_chrysomycin_KS02_angucyclineIKSb
griseusin_KS01_angucyclineIKSa_griseusin_KS02_angucyclineIKSb
azicemicin_KS01_angucyclineIKSa_azicemicin_KS02_angucyclineIKSb
saquayamycin_KS01_angucyclineIKSa_saquayamycin_KS02_angucyclineIKSb
chartreusin_KS01_aromaticKSa_chartreusin_KS02_aromaticKSb
chlortetracycline_KS01_tetracyclineKSa_chlortetracycline_KS02_tetracyclineKSb
oxytetracycline_KS01_tetracyclineKSa_oxytetracycline_KS02_tetracyclineKSb
SF2575_KS01_tetracyclineKSa_SF2575_KS02_tetracyclineKSb
chelocardin_KS01_tetracyclineKSa_chelocardin_KS02_tetracyclineKSb
aranciamycin_KS01_anthracyclineIKSa_aranciamycin_KS02_anthracyclineIKSb
mithramycin_KS01_anthracyclineIKSa_mithramycin_KS02_anthracyclineIKSb
chromomycin_KS01_anthracyclineIKSa_chromomycin_KS02_anthracyclineIKSb
polyketomycin_KS01_anthracyclineIKSa_polyketomycin_KS02_anthracyclineIKSb
nogalamycin_KS01_anthracyclineIKSa_nogalamycin_KS02_anthracyclineIKSb
steffimycin_KS01_anthracyclineIKSa_steffimycin_KS02_anthracyclineIKSb
kosinostatin_KS01_anthracyclineIKSa_kosinostatin_KS02_anthracyclineIKSb
erdacin_KS01_isochromanequinoneKSa_erdacin_KS02_isochromanequinoneKSb
granaticin_KS01_isochromanequinoneKSa_granaticin_KS02_isochromanequinoneKSb
naphthocyclinone_KS01_isochromanequinoneKSa_naphthocyclinone_KS02_isochromanequinoneKSb
actinorhodin_KS01_isochromanequinoneKSa_actinorhodin_KS02_isochromanequinoneKSb
enterocin_KS01_isochromanequinoneKSa_enterocin_KS02_isochromanequinoneKSb
R1128_KS01_isochromanequinoneKSa_R1128_KS02_isochromanequinoneKSb
alnumycin_KS01_isochromanequinoneKSa_alnumycin_KS02_isochromanequinoneKSb
frenolicin_KS01_isochromanequinoneKSa_frenolicin_KS02_isochromanequinoneKSb
aclacinomycin_KS01_anthracyclineIIKSa_aclacinomycin_KS02_anthracyclineIIKSb
cosmomycin_KS01_anthracyclineIIKSa_cosmomycin_KS02_anthracyclineIIKSb
doxorubicin_KS01_anthracyclineIIKSa_doxorubicin_KS02_anthracyclineIIKSb
lomaiviticin_KS01_angucyclineIIKSa_lomaiviticin_KS02_angucyclineIIKSb
nenestatin_KS01_angucyclineIIKSa_nenestatin_KS02_angucyclineIIKSb
hedamycin_KS01_aromaticKSa_hedamycin_KS02_aromaticKSb
griseorhodin_KS01_pentangularpolyphenolKSa_griseorhodin_KS02_pentangularpolyphenolKSb
rubromycin_KS01_pentangularpolyphenolKSa_rubromycin_KS02_pentangularpolyphenolKSb
lysolipin_KS01_pentangularpolyphenolKSa_lysolipin_KS02_pentangularpolyphenolKSb
xantholipin_KS01_pentangularpolyphenolKSa_xantholipin_KS02_pentangularpolyphenolKSb
fasamycin_KS01_pentangularpolyphenolKSa_fasamycin_KS02_pentangularpolyphenolKSb
arenimycin_KS01_pentangularpolyphenolKSa_arenimycin_KS02_pentangularpolyphenolKSb
pradimicin_KS01_pentangularpolyphenolKSa_pradimicin_KS02_pentangularpolyphenolKSb
A74528_KS01_pentangularpolyphenolKSa_A74528_KS02_pentangularpolyphenolKSb
fredericamycin_KS01_pentangularpolyphenolKSa_fredericamycin_KS02_pentangularpolyphenolKSb
benastatin_KS01_pentangularpolyphenolKSa_benastatin_KS02_pentangularpolyphenolKSb
FD594_KS01_pentangularpolyphenolKSa_FD594_KS02_pentangularpolyphenolKSb
elloramycin_KS01_tetracenomycinKSa_elloramycin_KS02_tetracenomycinKSb
tetracenomycin_KS01_tetracenomycinKSa_tetracenomycin_KS02_tetracenomycinKSb
lactonamycin_KS01_tetracenomycinKSa_lactonamycin_KS02_tetracenomycinKSb
gilvocarcin_KS01_aromaticKSa_gilvocarcin_KS02_aromaticKSb
StreptomycesavermitilisSP_KS01_sporepigmentKSa_StreptomycesavermitilisSP_KS02_sporepigmentKSb
StreptomycescollinusSP_KS01_sporepigmentKSa_StreptomycescollinusSP_KS02_sporepigmentKSb
StreptomycescoelicolorSP_KS01_sporepigmentKSa_StreptomycescoelicolorSP_KS02_sporepigmentKSb
resistomycin_KS01_aromaticKSa_resistomycin_KS02_aromaticKSb
0.22

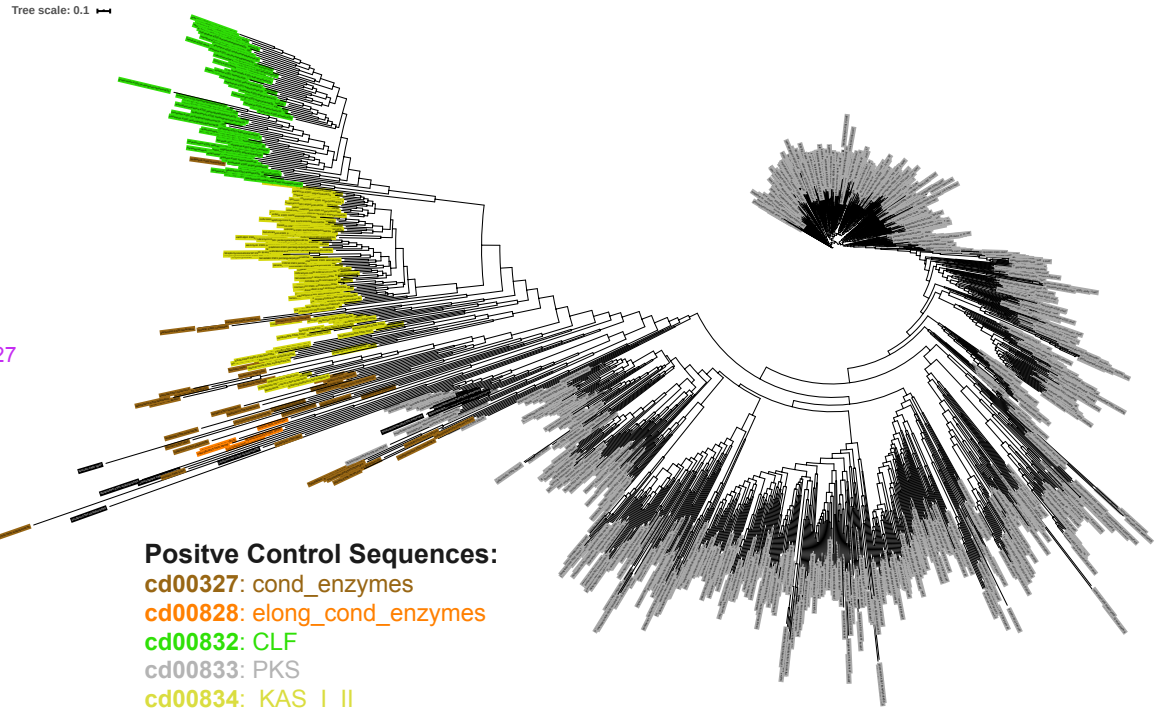
Figure S9. Maximum likelihood type II aromatic KS phylogeny of concatenated KSα and KSβ sequences from 59 biosynthetic gene clusters. The full name of each sequence is listed on the branch tips, which can help link a query match to a specific location in the tree. Bootstrap support is listed for each node.

Figure S10.

A. CDD **Condensing Enzyme Superfamily**: **634**

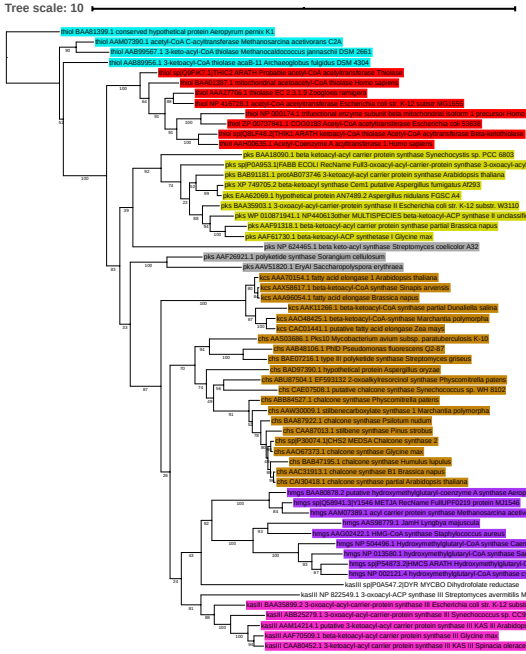


B. **NaPDoS2** database sequences: **1877**



Positve Control Sequences:
cd00327: cond_enzymes
cd00828: elong_cond_enzymes
cd00832: CLF
cd00833: PKS
cd00834: KAS_I_II
cl24048: Ketoacyl-synt_2
cl37173: omega_3_PfaA

C. **Jiang et al. 2008** tree: **49**



Negative Control Sequences:
cd00825: decarbox_cond_enzymes
cd00827: init_cond_enzymes
cd00830: KAS_III
cd00831: CHS_like
cd00826: nondecarbox_cond_enzymes
cd00751: thiolase
cd00829: SCP-x_thiolase

D. **Type III PKS** MIBiG 2.0: **14**

Accession	Main Product	Biosynthetic Class	Organism	KS Sequence
BGC0000189	3-(2'-hydroxy-3'-oxo-4'-methylpentyl)-indole 3-(2'-hydroxy-3'-oxo-4'-methylhexyl)-indole 3-(2'-acetoxy-3'-oxo-4'-methylpentyl)-indole 3-(2'-acetoxy-3'-oxo-4'-methylhexyl)-indole	Type III Polyketide	*Xenorhabdus bovienii* SS-2004	XBJ1_3898 XBJ1_3900
BGC0000285	flaviolin rhamnoside 3,3'-diflaviolin flaviolin	Type III Polyketide Oligosaccharide	*Saccharopolyspora erythraea*	rppA
BGC0001064	cylindrocyclophane D cylindrocyclophane E cylindrocyclophane F	Modular Type I Polyketide Type III Polyketide	*Cylindrospermum licheniforme* UTEX B 2014	AFV96140 AFV96143
BGC0001079	napyradiomycin A80915C	Terpene Type III Polyketide	*Streptomyces aculeolatus*	napT1 napB1
BGC0001083	merochlorin A, merochlorin B deschloro-merochlorin A, deschloro-merochlorin B isochloro-merochlorin B, dichloro-merochlorin B merochlorin D, merochlorin C	Terpene Type III Polyketide	*Streptomyces sp.* CNH189	mcl3 mcl4 mcl17
BGC0001150	pamamycin-607	Type II Polyketide Type III Polyketide	*Streptomyces alboniger*	pamG pamD pamE pamK

Figure S10. **Negative control KS sequence selection.** All sequence accession information is listed in Table S3.

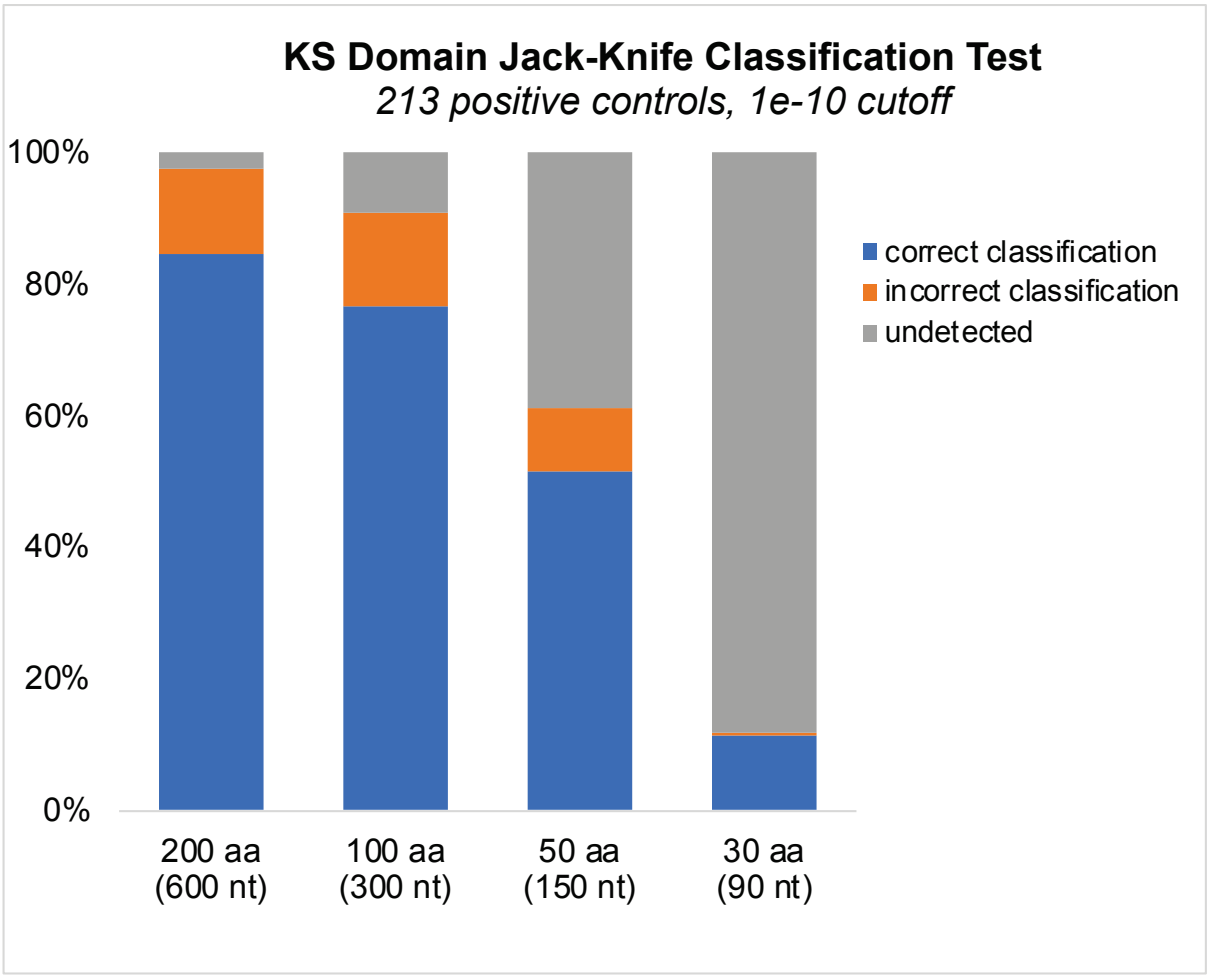
A) Distance matrix tree of 1,072 condensing enzyme superfamily (cl09938) sequences from the NCBI Conserved Domain Database[6] (CDD) tool CDtree[7], colored by conserved domain (CD) family in iTOL[8] (see panel B and C keys). Stars mark CD families not represented in the NaPDoS2 database, which were selected as negative controls (634 total). The negative control CD families comprise: initiating condensing enzymes (init_cond_enzymes, cd00827; n=84), chalcone and stilbene synthases (CHS_like, cd00831; n=67), decarboxylating condensing enzymes (decarbox_cond_enzymes, cd00825; n=3), ketoacyl-acyl carrier protein synthase III enzymes (KAS_III, cd00830; n=130), sterol carrier protein (SCP)-x isoform-associated thiolase domains (SCP-x_thiolase, cd00829; n=125), non-decarboxylating condensing enzymes (nondecarbox_cond_enzymes, cd00826; n=2), and thiolase enzymes (thiolase, cd00751; n=223).

B) Phylogenetic tree of the 1,877 NaPDoS2 KS sequences colored by CD family as determined by NCBI CD-Search[6] (see key). These sequences, which are associated with experimentally verified PKS and FAS biosynthetic gene clusters (BGCs), were selected as positive controls. The 1,877 NaPDoS2 KS sequences were aligned using MUSCLE[9]; the phylogenetic tree was calculated using FastTreeMP[10] on the CIPRES Science Gateway[11] and visualized in iTOL[8]. The NaPDoS2 database positive control CD families comprise: the condensing enzymes subfamily (cond_enzymes, cd00327; n=39), the elongating condensing enzyme subfamily (elong_cond_enzymes, cd00828; n=5), the chain-length factor subfamily (CLF, cd00832; n=70), the polyketide synthase PKS subfamily (PKS, cd00833; 1,649), the beta-ketoacyl-acyl carrier protein synthase (KAS) type I and II subfamily (KAS_I_II, cd00834; n=102), the N-terminal domain beta-ketoacyl synthase pfam13723 superfamily (cl24048; n=5), and the polyketide-type polyunsaturated fatty acid synthase omega-3 PfaA TIGR02813 superfamily (cl37173; n=7).

C) Phylogenetic tree of 61 condensing enzyme superfamily sequences from Jiang *et al* 2008[12], colored by conserved domain family as determined by NCBI CD-Search[6] (see panel B and C keys). Of these, 49 sequences (all but the 12 sequences colored yellow and grey) were added to the pool of negative control sequences. The 61 sequences were aligned using MUSCLE[9]; ProtTest 3.4.2[13] was used to define a model; the phylogeny was calculated with RAxML[14] WAG+G with 200 bootstraps; and the resulting tree visualized in iTOL[8].

D) Fourteen KS domains from the six experimentally characterized type III PKS BGCs in the MIBiG 2.0[5] repository were also added to the pool of negative control sequences. Sequence names are colored by their CD family as determined by NCBI CD-Search[6].

Figure S11.


KS Domain Jack-Knife Classification Test
213 positive controls, 1e-10 cutoff
100%
80%
60%
40%
20%
0%
200 aa (600 nt)
100 aa (300 nt)
50 aa (150 nt)
30 aa (90 nt)
correct classification
incorrect classification
undetected

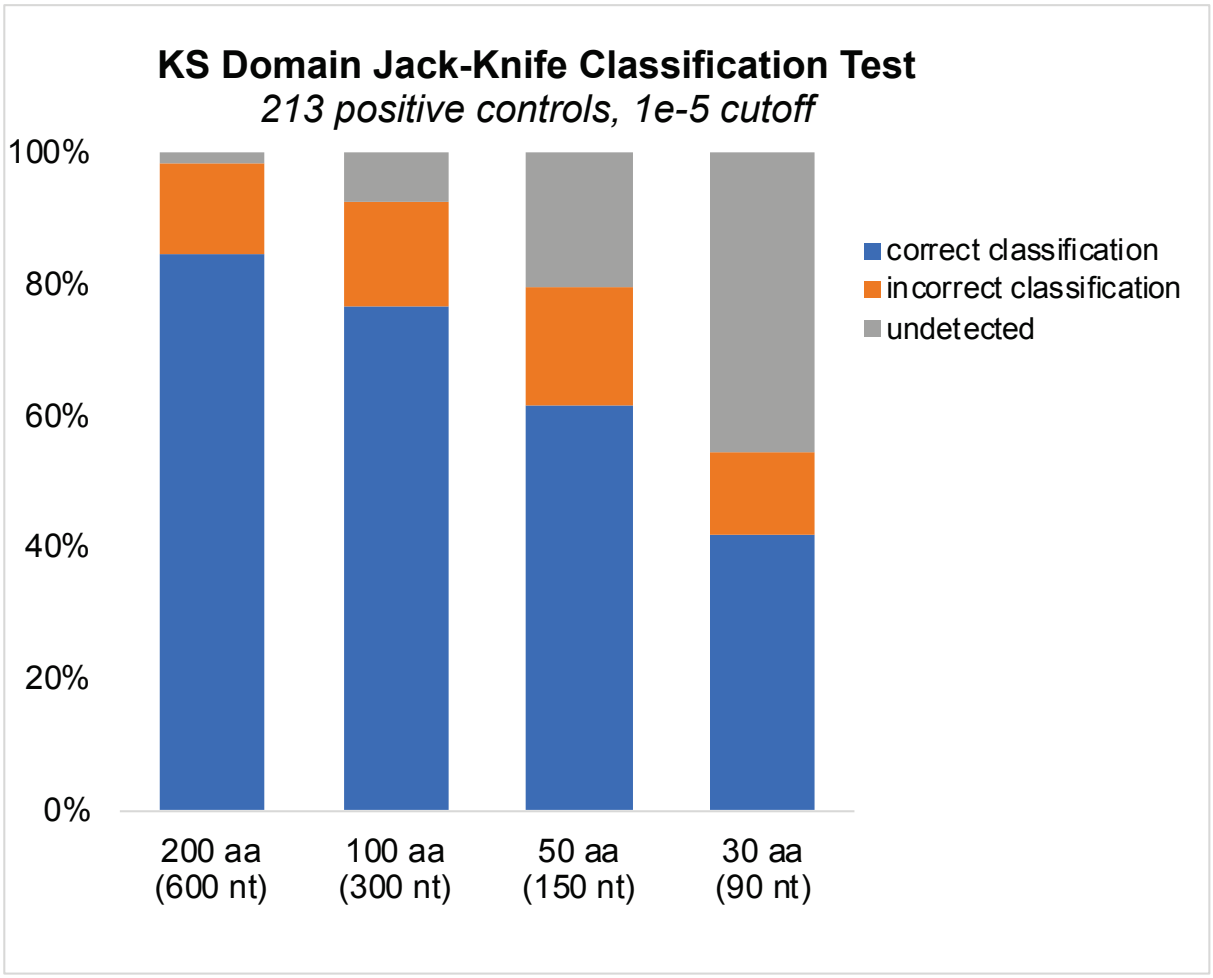

KS Domain Jack-Knife Classification Test
213 positive controls, 1e-5 cutoff
100%
80%
60%
40%
20%
0%
200 aa (600 nt)
100 aa (300 nt)
50 aa (150 nt)
30 aa (90 nt)
correct classification
incorrect classification
undetected

Figure S11. **Effect of query size on KS detection and classification accuracy at various E-values.** Classifications were based on varying BLASTP e-value cutoff scores as indicated for the closest non-self database match. Test sequences of varying lengths were obtained as overlapping sliding window subsequences covering the full length of 213 non-redundant, positive control KS domains.

Figure S12.

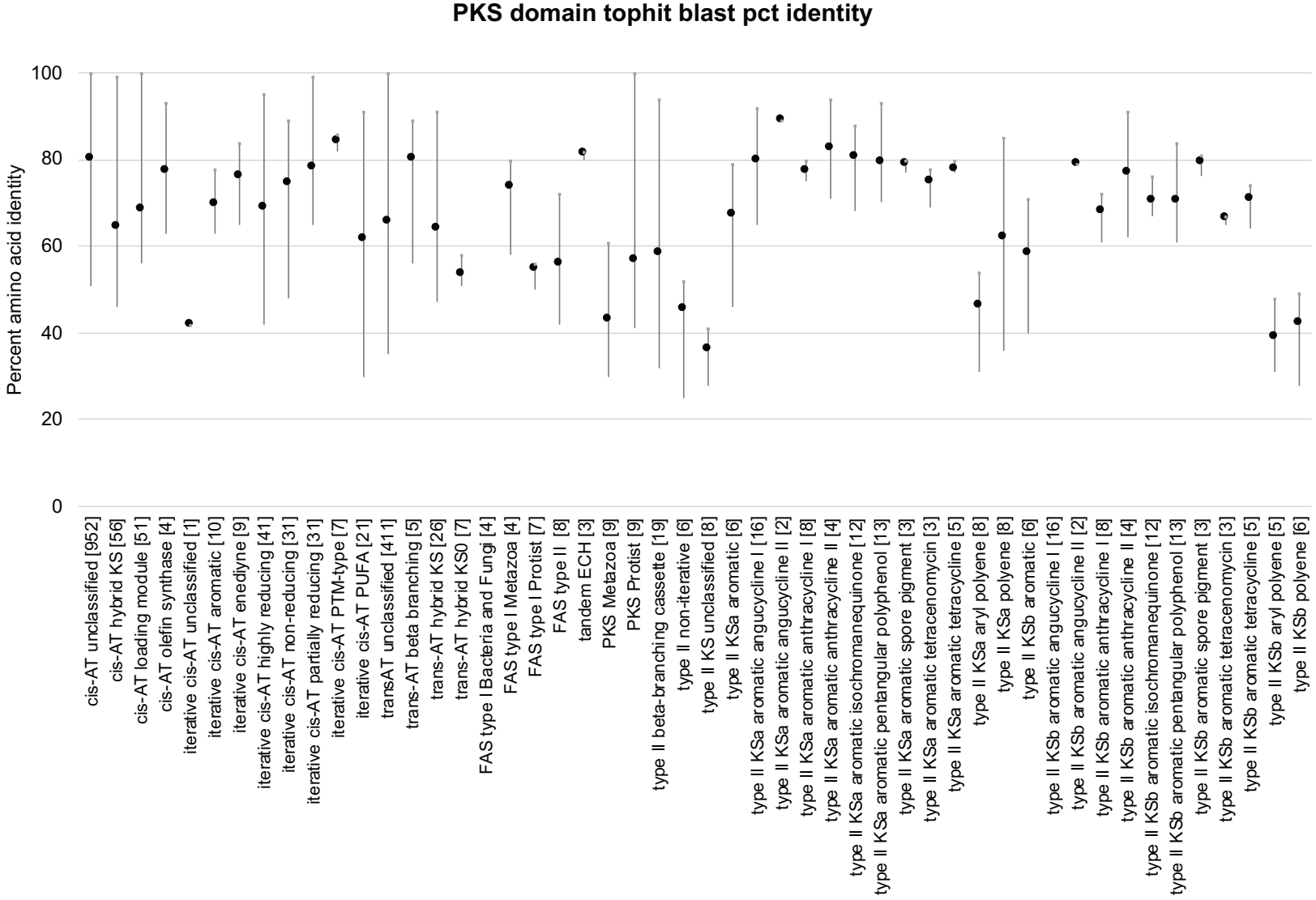
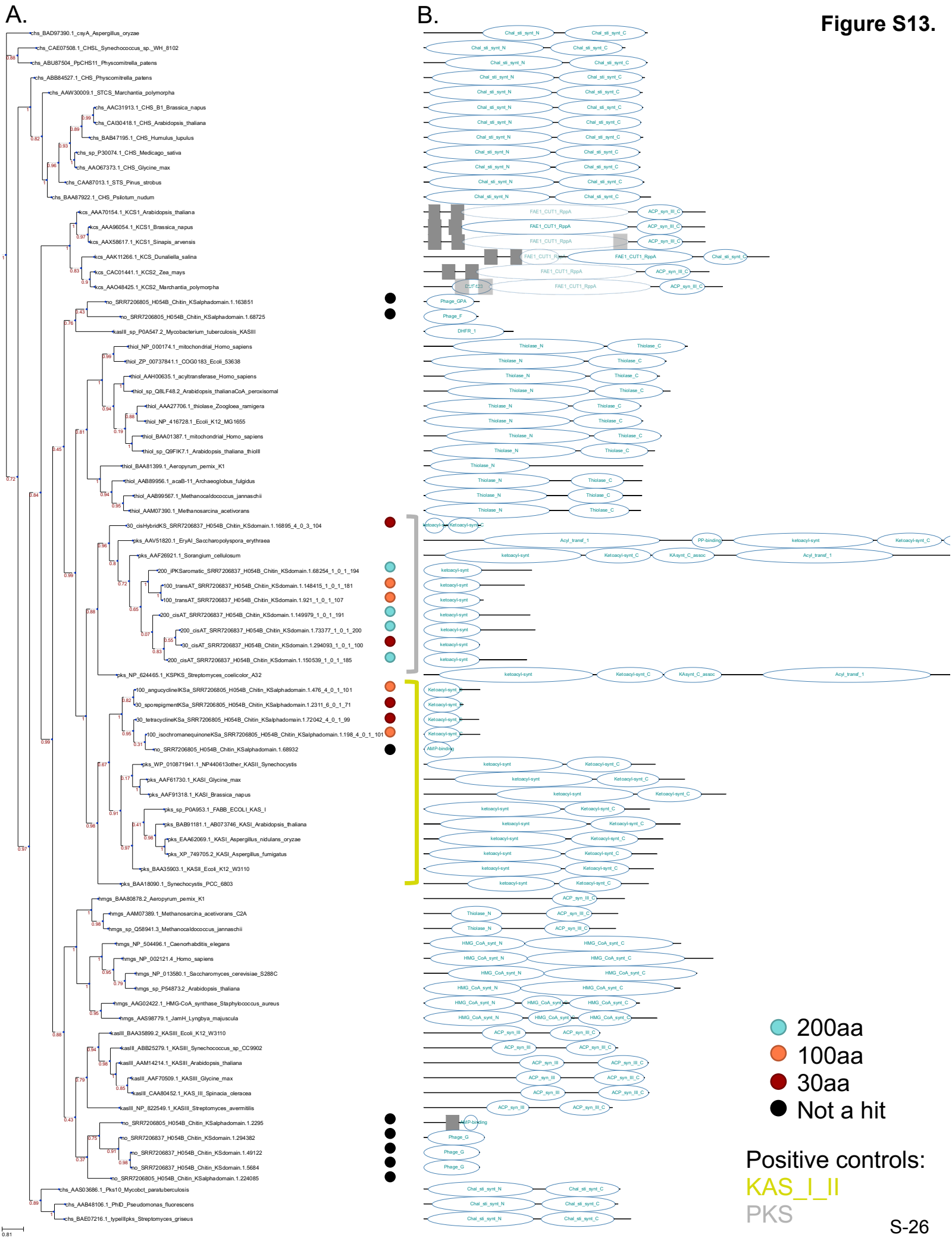

PKS domain tophit blast pct identity
Percent amino acid identity
100
80
60
40
20
0
cis-AT unclassified [952]
cis-AT hybrid KS [56]
cis-AT loading module [51]
cis-AT olefin synthase [4]
iterative cis-AT unclassified [1]
iterative cis-AT aromatic [10]
iterative cis-AT enediyne [9]
iterative cis-AT highly reducing [41]
iterative cis-AT non-reducing [31]
iterative cis-AT partially reducing [31]
iterative cis-AT PTM-type [7]
iterative cis-AT PUFA [21]
transAT unclassified [411]
trans-AT beta branching [5]
trans-AT hybrid KS [26]
trans-AT hybrid KS0 [7]
FAS type I Bacteria and Fungi [4]
FAS type I Metazoa [4]
FAS type I Protist [7]
FAS type II [8]
tandem ECH [3]
PKS Metazoa [9]
PKS Protist [9]
type II beta-branching cassette [19]
type II non-iterative [6]
type II KS unclassified [8]
type II KSa aromatic [6]
type II KSa aromatic angucycline I [16]
type II KSa aromatic angucycline II [2]
type II KSa aromatic anthracycline I [8]
type II KSa aromatic anthracycline II [4]
type II KSa aromatic isochromanequinone [12]
type II KSa aromatic pentangular polyphenol [13]
type II KSa aromatic spore pigment [3]
type II KSa aromatic tetracenomycin [3]
type II KSa aromatic tetracycline [5]
type II KSa aryl polyene [8]
type II KSa polyene [8]
type II KSb aromatic [6]
type II KSb aromatic angucycline I [16]
type II KSb aromatic angucycline II [2]
type II KSb aromatic anthracycline I [8]
type II KSb aromatic anthracycline II [4]
type II KSb aromatic isochromanequinone [12]
type II KSb aromatic pentangular polyphenol [13]
type II KSb aromatic spore pigment [3]
type II KSb aromatic tetracenomycin [3]
type II KSb aromatic tetracycline [5]
type II KSb aryl polyene [5]
type II KSb polyene [6]

Figure S12. KS domain sequence diversity. Percent sequence identities were determined for each KS class in the NaPDoS2 database using an all-against-all BLASTP comparison. Mean values (points) and ranges (vertical lines) are shown for top non-self matches within each NaPDoS2 class (x-axis). Bracketed values indicate the total number of database sequences in that class.

Figure S13.


A.
B.
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chs_CAE07508.1_CHSL_Synechococcus_sp._WH_8102
chs_ABU87504_PpCHS11_Physcomitrella_patens
chs_ABB84527.1_CHS_Physcomitrella_patens
chs_AAW30009.1_STCS_Marchantia_polymorpha
chs_AAC31913.1_CHS_B1_Brassica_napus
chs_CAB30418.1_CHS_Arabidopsis_thaliana
chs_BAB47195.1_CHS_Humulus_lupulus
chs_sp_P30074.1_CHS_Medicago_sativa
chs_AAO67373.1_CHS_Glycine_max
chs_CAA87013.1_STS_Pinus_strobus
chs_BAA87922.1_CHS_Psilotum_nudum
kcs_AAA70154.1_KCS1_Arabidopsis_thaliana
kcs_AAA96054.1_KCS1_Brassica_napus
kcs_AAX58617.1_KCS1_Sinapis_arvensis
kcs_AAK11266.1_KCS_Dunaliella_salina
kcs_CAC01441.1_KCS2_Zea_mays
kcs_AAO48425.1_KCS2_Marchantia_polymorpha
no_SRR7206805_H054B_Chitin_KSalphadomain.1.163851
no_SRR7206805_H054B_Chitin_KSalphadomain.1.68725
kasIII_sp_P0A547.2_Mycobacterium_tuberculosis_KASIII
thiol_NP_000174.1_mitochondrial_Homo_sapiens
thiol_ZP_00737841.1_COG0183_Ecoli_53638
thiol_AAH00635.1_acyltransferase_Homo_sapiens
thiol_sp_Q8LF48.2_Arabidopsis_thalianaCoA_peroxisomal
thiol_AAA27706.1_thiolase_Zoogloea_ramigera
thiol_NP_416728.1_Ecoli_K12_MG1655
thiol_BAA01387.1_mitochondrial_Homo_sapiens
thiol_sp_Q9FIK7.1_Arabidopsis_thaliana_thiolII
thiol_BAA81399.1_Aeropyrum_pernix_K1
thiol_AAB89956.1_acaB-11_Archaeoglobus_fulgidus
thiol_AAB99567.1_Methanocaldococcus_jannaschii
thiol_AAM07390.1_Methanosarcina_acetivorans
30_cisHybridKS_SRR7206837_H054B_Chitin_KSdomain.1.16895_4_0_3_104
pks_AAV51820.1_EryAI_Saccharopolyspora_erythraea
pks_AAF26921.1_Sorangium_cellulosum
200_iPKSaromatic_SRR7206837_H054B_Chitin_KSdomain.1.68254_1_0_1_194
100_transAT_SRR7206837_H054B_Chitin_KSdomain.1.148415_1_0_1_181
100_transAT_SRR7206837_H054B_Chitin_KSdomain.1.921_1_0_1_107
200_cisAT_SRR7206837_H054B_Chitin_KSdomain.1.149979_1_0_1_191
200_cisAT_SRR7206837_H054B_Chitin_KSdomain.1.73377_1_0_1_200
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pks_NP_624465.1_KSPKS_Streptomyces_coelicolor_A32
100_angucyclineIKSa_SRR7206805_H054B_Chitin_KSalphadomain.1.476_4_0_1_101
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no_SRR7206805_H054B_Chitin_KSalphadomain.1.68932
pks_WP_010871941.1_NP440613other_KASII_Synechocystis
pks_AAF61730.1_KASI_Glycine_max
pks_AAF91318.1_KASI_Brassica_napus
pks_sp_P0A953.1_FABB_ECOLI_KAS_I
pks_BAB91181.1_AB073746_KASI_Arabidopsis_thaliana
pks_EAA62069.1_KASI_Aspergillus_nidulans_oryzae
pks_XP_749705.2_KASI_Aspergillus_fumigatus
pks_BAA35903.1_KASII_Ecoli_K12_W3110
pks_BAA18090.1_Synechocystis_PCC_6803
hmgs_BAA80878.2_Aeropyrum_pernix_K1
hmgs_AAM07389.1_Methanosarcina_acetivorans_C2A
hmgs_sp_Q58941.3_Methanocaldococcus_jannaschii
hmgs_NP_504496.1_Caenorhabditis_elegans
hmgs_NP_002121.4_Homo_sapiens
hmgs_NP_013580.1_Saccharomyces_cerevisiae_S288C
hmgs_sp_P54873.2_Arabidopsis_thaliana
hmgs_AAG02422.1_HMG-CoA_synthase_Staphylococcus_aureus
hmgs_AAS98779.1_JamH_Lyngbya_majuscula
kasIII_BAA35899.2_KASIII_Ecoli_K12_W3110
kasIII_ABB25279.1_KASIII_Synechococcus_sp_CC9902
kasIII_AAM14214.1_KASII_Arabidopsis_thaliana
kasIII_AAF70509.1_KASIII_Glycine_max
kasIII_CAA80452.1_KAS_III_Spinacia_oleracea
kasIII_NP_822549.1_KASIII_Streptomyces_avermitilis
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no_SRR7206837_H054B_Chitin_KSdomain.1.49122
no_SRR7206837_H054B_Chitin_KSdomain.1.5684
no_SRR7206805_H054B_Chitin_KSalphadomain.1.224085
chs_AAS03686.1_Pks10_Mycobct_paratuberculosis
chs_AAB48106.1_PhlD_Pseudomonas_fluorescens
chs_BAE07216.1_typeIIIpks_Streptomyces_griseus
0.81
Chal_sti_synt_N
Chal_sti_synt_C
FAE1_CUT1_RppA
ACP_syn_III_C
DUF423
Phage_GPA
Phage_F
DHFR_1
Thiolase_N
Thiolase_C
ketoacyl-synt
Ketoacyl-synt_C
Acyl_transf_1
PP-binding
KAsynt_C_assoc
AMP-binding
ACP_syn_III
HMG_CoA_synt_N
HMG_CoA_synt_C
Phage_G
200aa
100aa
30aa
Not a hit
Positive controls:
KAS_I_II
PKS

Figure S13. **Amplicon detection accuracy.** A) Phylogenetic tree of 20 amplicons from Elfeki *et al.* 2018[15] (bolded) and 61 condensing enzyme superfamily sequences from Jiang *et al.* 2008[12]. The 20 amplicons from Elfeki *et al.* 2018[15] (bolded) were detected by NaPDoS2 at 30aa, 100aa, or 200aa minimum alignment lengths (red, orange, or teal circle, respectively) or not detected (not a "hit") at any alignment length (black circle). Grey and yellow brackets include positive control KS sequences as described in Figure S10. B) The conserved domains of all sequences as defined by TREND[16] (http://trend.zhulinlab.org/) from the NCBI conserved domain database are illustrated (gray squares are predicted transmembrane regions). The example "KS" amplicons not detected by NaPDoS2 are off-target amplifications with conserved domains relating to phage proteins. The KS amplicons detected by NaPDoS2 cluster within the PKS and KAS_I_II conserved domain positive control sequences from type I and II PKSs, respectively.

Abbreviations in (A) taxa branches from Jiang *et al.* 2008[12] are as listed in Figure S10; sequences from Elfeki *et al.* 2018[15] are annotated with the minimum amino acid alignment length setting/or "no" for "not a hit", NaPDoS2 classification, SRA dataset, sample information, and NaPDoS2 domain range.
Abbreviations in (B) conserved domains as defined by TREND[16].

Table S1.

Table S1. Typical NaPDoS pipeline processing times for genomic, PCR amplicon, and metagenomic data sets for the original NaPDoS release (V1) and NaPDoS2 (V2). Initial data upload times are not included, as they vary widely according to internet connection speed. Matches were analyzed using a minimum alignment length of 200 amino acids for all data sets except the 243 nt amplicons, which were screened using a 50 amino acid cutoff value. Dashes indicate missing values for queries that could not be analyzed in NaPDoS version 1 because they exceeded maximum size limitations (50,000 sequences or 30 MB file size). Limits in NaPDoS Version 2 have been increased to 500,000 sequences or 500 MB file size.

Data set	Type	Acession numbers	File size (MB)	Input seqs	V1 sec	V2 sec	Num. domain matches
Salinispora arenicola CNS-205, complete genome	nucleic acid	GCF_000018265.1	5.9	1	106	10	33
Aplysina aerophoba assembed metagenome - random subsample	nucleic acid (assembled contigs)	IMG 3300002222	32.9	1,250	192	13	32
Aplysina aerophoba assembed metagenome - random subsample	nucleic acid (assembled contigs)	IMG 3300002222	46.1	2,500	276	19	38
Aplysina aerophoba assembed metagenome - random subsample	nucleic acid (assembled contigs)	IMG 3300002222	82.5	10,000	-	33	62
Aplysina aerophoba assembed metagenome - random subsample	nucleic acid (assembled contigs)	IMG 3300002222	106.4	20,000	-	42	72
Aplysina aerophoba assembed metagenome - random subsample	nucleic acid (assembled contigs)	IMG 3300002222	141	49,000	-	57	87
Aplysina aerophoba assembed metagenome	nucleic acid (assembled contigs)	IMG 3300002222	211.4	219,427	-	91	94
Salinispora arenicola CNS-205, complete genome	predicted protein	GCF_000018265.1	2.0	4,820	22	6	33
23 draft bacterial genomes (MAGs) - random subsample	predicted protein	PRJNA320446	9.5	25,000	79	4	16
23 draft bacterial genomes (MAGs) - random subsample	predicted protein	PRJNA320446	13.4	35,000	107	6	20
23 draft bacterial genomes (MAGs) - random subsample	predicted protein	PRJNA320446	19.1	50,000	149	7	31
23 draft bacterial genomes (MAGs)	predicted protein	PRJNA320446	28.1	73,530	216	9	49
Aplysina aerophoba assembed metagenome	predicted protein	IMG 3300002222	70.9	365,131	-	41	94
S31 Antarctic soil, KS amplicons - random subsample	nucleic acid amplicon (243 nt)	ERR1527879	1.5	5,000	50	8	3,194
S31 Antarctic soil, KS amplicons	nucleic acid amplicon (243 nt)	ERR1527879	6.5	19,909	198	36	14,024

Table S2. NaPDoS2 database summary. Table lists the number of KS domains and their phylum level assignments for each class and subclass.

Table S2.

Class	Subclass		Bacteria	Fungi	Other Eukaryota	Total (across all taxa)
Polyketide Synthases						
type I modular *cis* -AT	olefin synthase		4			4
	loading module		51			51
	hybrid KS		56			56
	tandem ECH		3			3
	no subclass		952			952
		Total	**1066**	**0**	**0**	**1066**
type I iterative *cis* -AT	PUFA (polyunsaturated fatty acids)		18		3	21
	enediynes		9			9
	aromatic		10			10
	PTM-type (polycyclic tetramate macrolactam)		7			7
	non-reducing			31		31
	partially reducing			7		7
	highly reducing			41		41
	no subclass		1			1
		Total	**45**	**79**	**3**	**127**
type I *trans* -AT	beta-branching module		5			5
	hybrid KS		19			19
	hybrid KS0 (non-elongating KS)		7			7
	no subclass		411			411
		Total	**442**	**0**	**0**	**442**
type I Metazoa-type PKS	no subclass				9	9
type I Protist-type PKS	no subclass				9	9
		Total	**0**	**0**	**18**	**18**
		Total	**1553**	**79**	**21**	**1653**
type II aromatic	angucycline-derived I		32			32
	angucycline-derived II		4			4
	anthracycline-derived I		16			16
	anthracycline-derived II		8			8
	isochromanequinone-derived		24			24
	pentangular polyphenol-derived		26			26
	tetracenomycin-derived		6			6
	tetracycline-derived		10			10
	spore pigment		6			6
	unclassified		12			12
		Total	**144**	**0**	**0**	**144**
type II beta-branching cassettes	no subclass		19			19
type II polyenes	KSa, KSb		14			14
type II aryl polyenes	KSa, KSb		13			13
type II non-iterative	no subclass		6			6
type II unclassified	no subclass		5			5
		Total	**57**	**0**	**0**	**57**
		Total	**201**	**0**	**0**	**201**
Fatty acid synthases						
type I FAS	Bacterial-Fungal-type		2	2		4
	Metazoan-type				4	4
	Protist-type				7	7
		Total	**2**	**2**	**11**	**15**
type II FAS	no subclass		8			8
		Total	**8**	**0**	**0**	**8**
		Total	**10**	**2**	**11**	**23**
		Grand Total	**1764**	**81**	**32**	**1877**

Table S3. **Accession numbers for negative controls, application use cases, type II aromatic KS sequences, example structures, and associated references.**

The first tab “Negative Control Sequences” lists the accession numbers for the 697 negative control sequences listed by source: NCBI Conserved Domain family outside, Jiang *et al.* 2008[12] tree, and MIBiG 2.0[5] type III PKS BGCs.

The second tab “Application Use Case Accessions” lists the sequence/dataset accession numbers for the application use cases (listed by biological source type, data type, and relevant reference).

The third tab “TypeIIAromaticKS_ AccessNum_Refs” lists the GenBank protein ID for alpha and beta sequences, together with literature references used for the biosynthetic annotations in Figure 4 (i.e. poly-beta-keto chain length, starting unit, C-C cyclisation position).

The fourth tab "Example Structures Metadata" lists the PubMed ID/doi for the structures shown in Figures 2 and 5 along with the KS class/subclass associated with its biosynthesis, the chemical name, the BGC product name as reported in the NaPDoS2 BGCs tab, and the source species.

(provided as a Microsoft Excel file).

Table S4. ***Salinispora* spp. type II KS domains identified by NaPDoS version 1 (NaPDoS1)[1] and NaPDoS2.** 118 *Salinispora* genomes[17] were analyzed using the following default settings: NaPDoS1: HMM 1e-5 cutoff, 200aa minimum alignment length, pathway assignment BLASTP e-value 1e-5 cutoff; NaPDoS2: e-value cutoff 1e-8 and 200aa minimum alignment length. The NaPDoS1 output is limited to the total number of type II KSs detected. The NaPDoS2 output includes the total number of type II KSs ("Total NP2") and their subclassification. KSα and KSβ sequences are grouped together.

Abbreviations: Betabranch= type II beta-branching cassettes; Angl-I= type II aromatic angucycline-derived I; Angl-II= type II aromatic angucycline-derived II; Anth-I= type II aromatic anthracycline-derived I; Isochrom= type II aromatic isochromanequinone-derived; Polyphen= type II aromatic pentangular polyphenol-derived; Unclass= type II aromatic unclassified; Type II-uc= type II unclassified no subclass.

Table S4.

		NaPDoS1	NaPDoS2									
						Aromatic						
Species	**# genomes**	Type II	*Total NP2*	Betabranch	Polyene	Angl-I	Angl-II	Anth-I	Isochrom	Polyphen	Unclass	Type II-uc
S. tropica	12	48	108	-	60	-	24	-	-	24	-	-
S. fenicalii	2	12	24	-	12	4	4	-	-	4	-	-
S. cortesiana	1	4	5	-	1	-	2	-	-	2	-	-
S. mooreana	3	8	15	-	7	-	-	-	2	6	-	-
S. oceanensis	12	44	44	-	-	8	-	6	4	26	-	-
S. goodfellowii	1	4	5	-	1	-	2	-	-	2	-	-
S. vitiensis	3	12	28	-	14	-	6	-	-	6	-	2
S. pacifica	23	97	126	-	27	-	46	-	4	46	-	3
S. arenicola	61	134	307	2	173	-	-	4	-	126	2	-

Table S5. **Complete list of *Salinispora* spp. KS domains identified by NaPDoS2.** 118 *Salinispora* genomes[17] were analyzed using default settings (e-value cutoff 1e-8 and 200aa minimum alignment length). Table lists the number of KSs detected and their classification by strain. The summed total number of KSs found in each strain is listed as "NP2 Total". KSα and KSβ sequences are grouped together.

Abbreviations: Betabranch= type II beta-branching cassettes; Angl-I= type II aromatic angucycline-derived I; Angl-II= type II aromatic angucycline-derived II; Anth-I= type II aromatic anthracycline-derived I; Isochrom= type II aromatic isochromanequinone-derived; Polyphen= type II aromatic pentangular polyphenol-derived; Unclass= type II aromatic unclassified; Type II-uc= type II unclassified no subclass; *cis*-AT= type I modular *cis*-AT; cisloading= type I modular *cis*-AT loading module; cisHybridKS= type I modular *cis*-AT hybrid KS; Ened= type I iterative *cis*-AT enediynes; iPKSaromatic= type I iterative *cis*-AT aromatic; PTM= type I iterative *cis*-AT PTM-type (polycyclic tetramate macrolactam); *trans*-AT= type I *trans*-AT no subclass; *trans*-hybridKS0= type I *trans*-AT hybrid KS0 (non-elongating KS).

Table S5.

			NaPDoS2: KS classes																
			FAS	Type II PKS									Type I PKS						
						Aromatic								*cis*-AT modular		*cis*-AT iterative			*trans*-AT	
Species	***Strain***	***NP2 Total***	FAS II	Betabranch	Polyene	Angl-I	Angl-II	Anth-I	Isochrom	Polyphen	Unclass	TypeII-uc	*cis*-AT	cisloading	cisHybridKS	Ened	iPKSaromatic	PTM	*trans*-AT	*trans*-hybridKS0
S. tropica	CNB440	28	2		5		2			2			12	1	2	2				
S. tropica	CNB476	22	2		5		2			2			6	1	3	1				
S. tropica	CNB536	29	3		5		2			2			12	1	3	1				
S. tropica	CNH898	27	4		5		2			2			10	1	2	1				
S. tropica	CNR699	24	2		5		2			2			8	1	2	2				
S. tropica	CNS197	24	2		5		2			2			8	1	2	2				
S. tropica	CNS416	24	2		5		2			2			9	1	2	1				
S. tropica	CNT250	24	2		5		2			2			8	1	2	2				
S. tropica	CNT261	27	3		5		2			2			8	1	5	1				
S. tropica	CNY012	24	3		5		2			2			8	1	2	1				
S. tropica	CNY678	24	2		5		2			2			8	1	2	2				
S. tropica	CNY681	24	2		5		2			2			8	1	2	2				
S. fenicalii	CNT569	30	2		6	2	2			2			2	1	2	2			9	
S. fenicalii	CNR942	29	2		6	2	2			2			2	1	2	1			9	
S. cortesiana	CNY202	13	4		1		2			2			2		1	1				
S. mooreana	CNY646	24	5		5					2			7	2	2	1				
S. mooreana	CNS237	48	4		1					2			34	3	2	2				
S. mooreana	CNT150_DSM45549	13	4		1				2	2			2			2				
S. oceanensis	CNT854	10	4							2			1		1	1	1			
S. oceanensis	CNT584	13	4			4				2			1		1	1				
S. oceanensis	CNT124	13	4			4				2			1		1	1				
S. oceanensis	CNT138_DSM45547	30	4							4			17	1	2	1			1	
S. oceanensis	CNT029	9	4							2			1		1	1				
S. oceanensis	CNY703	9	4							2			1		1	1				
S. oceanensis	CNY673	11	4					2		2			1		1	1				
S. oceanensis	CNT045	21	6							2			4	4	3	1	1			
S. oceanensis	CNS996	23	6					2		2			4	5	3	1				
S. oceanensis	CNT403	16	5					2		2			2	2	2	1				
S. oceanensis	CNS860	15	5						2	2			1	2	2	1				
S. oceanensis	CNS863_DSM45543	16	5						2	2			2	2	2	1				
S. goodfellowii	CNY666	50	2		1		2			2			38	1	2	1			1	
S. vitiensis	CNS055	20	1		4		2			2			9		1	1				
S. vitiensis	CNT148_DSM45548	15	3		5		2			2		1	1		1					
S. vitiensis	CNS801	15	3		5		2			2		1	1		1					
S. pacifica	CNH732	20	2		1		2			2			9		3	1				
S. pacifica	CNQ768	19	2		1		2			2			8		3	1				
S. pacifica	CNR114	20	2		1		2			2			10	1	1	1				
S. pacifica	CNR510	23	2		1		2			2			11	1	3	1				
S. pacifica	CNR894	20	2		1		2			2			9		3	1				
S. pacifica	CNR909	21	3		1		2			2			10		1	1	1			
S. pacifica	CNS103	23	3		1		2			2			11	1	2	1				
S. pacifica	CNT001	23	2		1		2			2			11	1	3	1				
S. pacifica	CNT003	28	3		1		2			2			16	1	1	1			1	
S. pacifica	CNT084	28	4		1		2			2			14	2	1	1	1			
S. pacifica	CNT131	30	2		1		2			2			17	1	3	1			1	
S. pacifica	CNT133	29	4		1		2			2			15	2	1	1	1			
S. pacifica	CNT603	20	2		1		2			2			9		3	1				
S. pacifica	CNT609	26	3		1		2			2		1	13	2	1	1				
S. pacifica	CNT796	21	2		1		2		2	2			10		1	1				
S. pacifica	CNT851	20	2		1		2		2	2			9		1	1				
S. pacifica	CNT855	21	2		2		2			2		1	10		1	1				
S. pacifica	CNY239	20	2		1		2			2			8		4	1				
S. pacifica	CNY330	32	2		1		2			2			23		1	1				
S. pacifica	CNY331	37	2		2		2			2			26	1	1	1				
S. pacifica	CNY363	45	2		2		2			2			33	1	1	1			1	
S. pacifica	CNY498	22	2		1		2			2			10		3	1			1	
S. pacifica	CNS960_DSM45544	28	2		2		2			2		1	14		4	1				
S. arenicola	CNB458	24	2		2					2			13		1	2	2			
S. arenicola	CNB527	29	2		2					4			16		1	2	2			
S. arenicola	CNH643	36	3		4					2			21	1	2	2	1			
S. arenicola	CNH646	21	3		4					2			7		2	2	1			
S. arenicola	CNH713	34	3		4					2			19	1	2	2	1			
S. arenicola	CNH718	33	2		2					2			20	1	1	2	2	1		
S. arenicola	CNH877	29	3		4					2			15		2	2	1			
S. arenicola	CNH905	28	3		4					2			15		1	2	1			
S. arenicola	CNH941	23	3		4					2			7	1	3	2	1			
S. arenicola	CNH962	17	2		2					2			8			2	1			
S. arenicola	CNH963	18	2		2					2			9			2	1			
S. arenicola	CNH964	31	3	1	4					2			7	1	3	2	1		6	1
S. arenicola	CNH996B	26	3		2					2			13	1		2	2	1		
S. arenicola	CNH996	26	3		2					2			13	1		2	2	1		
S. arenicola	CNP105	31	3	1	4					2			7	1	3	2	1		6	1
S. arenicola	CNP193	23	3		4					2			7	1	3	2	1			
S. arenicola	CNQ748	38	2		2					2			24	1	2	2	2	1		
S. arenicola	CNQ884	50	2		2					2			37	2	1	2	2			
S. arenicola	CNR107	22	2		3					2			8		2	2	2	1		
S. arenicola	CNR425	33	2		2					2			20	1	2	2	2			
S. arenicola	CNR921	15	2		2					2			4		1	2	2			
S. arenicola	CNS051	16	2		2					2			5		1	2	2			
S. arenicola	CNS205	33	2		2					2			20	1	1	2	2	1		

Table S5.

			FAS	Type II PKS										Type I PKS							
		NP2				Aromatic								*cis*-AT modular		*cis*-AT iterative			*trans*-AT	
Species	***Strain***	***Total***	FAS II	Betabranch	Polyene	Angl-I	Angl-II	Anth-I	Isochrom	Polyphen	Unclass	TypeII-uc	*cis*-AT	cisloading	cisHybridKS	Ened	iPKSaromatic	PTM	*trans*-AT	*trans*-hybridKS0
S. arenicola	CNS243	50	2		2					2			36	2	1	2	2	1		
S. arenicola	CNS296	39	2		2					2			24	2	2	2	2	1		
S. arenicola	CNS299	35	2		2					2			22	2	1	2	2			
S. arenicola	CNS325	35	2		2					2	2		19	2	2	2	2			
S. arenicola	CNS342	32	2		2					2			20	1	1	2	2			
S. arenicola	CNS673	28	2		2					2			15		3	2	2			
S. arenicola	CNS744	27	2		2					4			12		2	2	2	1		
S. arenicola	CNS820	34	2		2					2			20	1	2	2	2	1		
S. arenicola	CNS848	38	3		4					2			20	3	3	2	1			
S. arenicola	CNT005	28	2		2					2			16	1	1	2	2			
S. arenicola	CNT798	28	3		4					2			14		2	2	1			
S. arenicola	CNT799	34	3		4					2			19	1	2	2	1			
S. arenicola	CNT800	30	3		4					2			16		2	2	1			
S. arenicola	CNT849	29	3		4					2			15		2	2	1			
S. arenicola	CNT850	27	3		4					2			13		2	2	1			
S. arenicola	CNT857	30	3		4					2			16		2	2	1			
S. arenicola	CNT859	29	3		4					2			16		1	2	1			
S. arenicola	CNX481	20	2		2					2			8		1	2	2	1		
S. arenicola	CNX482	19	2		2					2			7		1	2	2	1		
S. arenicola	CNX508	28	2		2					2			16		1	2	2	1		
S. arenicola	CNX814	21	2		2			2		2			7		1	2	2	1		
S. arenicola	CNX891	20	2		2			2		2			6		1	2	2	1		
S. arenicola	CNY011	30	3		4					2			16		2	2	1			
S. arenicola	CNY230	40	2		6					2			22	2	2	2	2			
S. arenicola	CNY231	30	2		2					2			18	1	1	2	2			
S. arenicola	CNY234	25	2		2					2			14		1	2	2			
S. arenicola	CNY237	25	2		2					2			13		1	2	2	1		
S. arenicola	CNY244	32	2		2					2			19	1	1	2	2	1		
S. arenicola	CNY256	23	2		2					2			12		1	2	2			
S. arenicola	CNY260	27	2		2					2			15		1	2	2	1		
S. arenicola	CNY280	40	3		8					2			19	2	3	2	1			
S. arenicola	CNY282	23	2		2					2			12		1	2	2			
S. arenicola	CNY486	24	2		2					2			13	1		2	2			
S. arenicola	CNY679	36	3		4					2			21	1	2	2	1			
S. arenicola	CNY685	31	2		2					2			19	1	1	2	2			
S. arenicola	CNY690	30	2		2					2			18	1	1	2	2			
S. arenicola	CNY694	30	2		2					2			18	1	1	2	2			
S. arenicola	CNS991_DSM45545	40	3		4					2			24	2	2	2	1			
	total	*3103*	*313*	*2*	*295*	*12*	*84*	*10*	*10*	*242*	*2*	*5*	*1490*	*92*	*200*	*186*	*104*	*18*	*36*	*2*

Table S6. **KS domains identified in 27 fungal genomes by NaPDoS2.** Table lists the total number of KSs detected using default settings (e-value cutoff 1e-8 and 200aa minimum alignment length) and their class and subclass distributions. The summed total number of KSs found in each genome is listed as “Total NP2”. The number of protein sequences in each analyzed genome file is listed as “# protein seq”.

Abbreviations: bfFASI= type I FAS Bacterial-Fungal-type; FASII= type II FAS; *cis*-AT= type I modular *cis*-AT; cisHybridKS= type I modular *cis*-AT hybrid KS; PR= type I iterative *cis*-AT partially reducing; NR= type I iterative *cis*-AT non-reducing; HR= type I iterative *cis*-AT highly reducing.

Table S6.

					Type I PKS				
					cis-AT modular		*cis*-AT iterative		
Fungal genome	**# protein seq**	*Total NP2*	bfFASI	FASII	*cis*-AT	cisHybridKS	PR	NR	HR
Aspergillus niger ATCC 1015	12,885	50	5	1	1	-	1	8	34
Aspergillus sergii CBS 130017	13,713	39	5	1	-	-	1	12	20
Aspergillus nidulans FGSC A4	9,556	37	5	1	1	-	-	14	16
Penicillium rolfsii F1880	9,955	26	2	1	-	-	-	6	17
Penicillium rubens Wisconsin 54-1255	12,791	25	2	1	-	-	2	3	17
Sclerotinia sclerotiorum 1980 UF-70	14,490	20	1	1	-	-	-	4	14
Leptosphaeria maculans JN3	12,469	17	2	1	-	-	-	4	10
Nectria haematococca MPVI isolate 77-13-4	15,708	15	1	1	-	-	-	2	11
Alternaria alternata SRC1lrK2f	13,466	14	1	1	-	1	-	3	8
Zymoseptoria tritici IPO323	10,941	14	1	1	-	-	-	2	10
Neurospora crassa OR74A	10,812	11	2	1	-	-	-	1	7
Arthrobotrys oligospora TWF154	13,042	9	1	1	-	-	1	-	6
Ustilago maydis 521	6,782	5	2	-	-	-	-	3	-
Allomyces macrogynus ATCC 38327	19,447	4	2	2	-	-	-	-	-
Laccaria bicolor S238N-H82	18,215	3	1	-	1	-	-	1	-
Mucor circinelloides 1006PhL	12,227	3	2	1	-	-	-	-	-
Coprinopsis cinerea okayama 7#130 CC3	13,356	2	1	-	-	-	-	1	-
Pyronema omphalodes CBS 100304	13,367	3	1	1	-	-	-	1	-
Schizophyllum commune H4-8	13,193	3	2	-	-	-	-	1	-
Batrachochytrium dendrobatidis JAM81	8,677	2	1	1	-	-	-	-	-
Debaryomyces hansenii CBS767	6,286	2	1	1	-	-	-	-	-
Neolecta irregularis DAH-3	5,579	2	1	1	-	-	-	-	-
Puccinia graminis tritici CRL 75-36-700-3	15,979	2	2	-	-	-	-	-	-
Saccharomyces cerevisiae S288C	6,002	2	1	1	-	-	-	-	-
Saitoella complicata NRRL Y-17804	7,023	2	1	1	-	-	-	-	-
Schizosaccharomyces pombe 972h-	5,132	2	1	1	-	-	-	-	-
Malassezia globosa CBS 7966	4,286	1	-	-	1	-	-	-	-

Table S7. **KS detection using NaPDoS versions 1 and 2.**
MIBiG Fungal PKS: NaPDoS2 detected all of the KS domains in 159 MIBiG 2.0[5] fungal PKS BGCs. NaPDoS version 1 and 2 analyses were run with the default settings.
Wawrik 2005 KS clones: While both NaPDoS versions 1 and 2 detected all 147 type II KS amplicons from Wawrik *et al*. 2005[18], NaPDoS2 could further delineate these sequences into three subclasses. NaPDoS version 1 and 2 analyses were run with the following settings: NaPDoS version 1: HMM 1e-5, 200aa minimum alignment length, pathway assignment: e-value cutoff of 1e-5; NaPDoS2: e-value cutoff 1e-8 and 50aa minimum alignment length. The summed total number of KSs found in each genome is listed as "Total NP1" and "Total NP2" for NaPDoS versions 1 and 2, respectively.

Abbreviations: Iter= Iterative type I; FA= fatty acid synthase; bfFASI= type I FAS Bacterial-Fungal-type; *cis*-AT= type I modular *cis*-AT; PR= type I iterative *cis*-AT partially reducing; NR= type I iterative *cis*-AT non-reducing; HR= type I iterative *cis*-AT highly reducing; Aro-KSα= type II aromatic unclassified KSα; Angl-I-KSα= type II aromatic angucycline-derived I KSα; Polyphen-KSα= type II aromatic pentangular polyphenol-derived KSα.

Table S7.

	NaPDoS1				**NaPDoS2**		Type I PKS				Type II PKS		
							cis-AT modular	*cis*-AT iterative			Aromatic		
Sequence collection	*Total NP1*	Type II	Iter	FA	*Total NP2*	bfFASI	*cis*-AT	PR	NR	HR	Aro-KSα	Angl-I-KSα	Polyphen-KSα
MIBiG Fungal PKS (159 BGCs)	14	-	7	7	182	10	4	7	71	90	-	-	-
Wawrik 2005 KS clones (147 seqs)	147	147	-	-	147	-	-	-	-	-	1	45	101

Table S8. **NaPDoS2 analysis of the *Elysia chlorotica* genome.** NaPDoS2 identified nine KSs from the *Elysia chlorotica* CDSs (fna), protein (faa), and translated CDSs (faa) genomes[19]. Highlighted KSs were identified in Torres *et al*. 2020[20] as being associated with new FAS-like animal PKSs (EcPKS1, EcPKS2) and an FAS (EcFAS). The analyses were run with NaPDoS2 default settings (e-value cutoff 1e-8 and 200aa minimum alignment length).

Table S8.

Match	Query ID	Database match ID	% ID	Align length	E-value	BGC Match	Domain Class	Domain Subclass
EcFAS	RUS90834.1	OryziasFAS_KS01_MetazoaFASI	60	403	5.7e-152	Oryzias latipes FAS	type I FAS	Metazoan-type
EcPKS1	RUS71288.1	HomoFAS_KS01_MetazoaFASI	52	406	9.6e-119	Homo sapiens FAS	type I FAS	Metazoan-type
	RUS77019.1	OryziasFAS_KS01_MetazoaFASI	49	403	2.7e-117	Oryzias latipes FAS	type I FAS	Metazoan-type
EcPKS2	RUS75294.1	OryziasFAS_KS01_MetazoaFASI	49	403	4.7e-117	Oryzias latipes FAS	type I FAS	Metazoan-type
	RUS92164.1	MelopsittacusFAS_KS01_MetazoaFASI	39	410	2.9e-76	Melopsittacus undulatus FAS	type I FAS	Metazoan-type
	RUS75295.1	OryziasFAS_KS01_MetazoaFASI	45	308	6.3e-73	Oryzias latipes FAS	type I FAS	Metazoan-type
	RUS68442.1	AliivibrioAPE_KS03_FASII	60	407	1.3e-137	Aliivibrio fischeri aryl polyene	type II FAS	no subclass
	RUS69056.1	EscherichiaFAS_KS02_FASII	57	420	1.9e-136	Escherichia coli FAS	type II FAS	no subclass
	RUS77541.1	EscherichiaFAS_KS02_FASII	47	421	4.8e-103	Escherichia coli FAS	type II FAS	no subclass

Table S9. **Moorea sediment metagenomes analyzed with NaPDoS2.** KS domains from 20 marine sediment metagenomes[21] were assigned to 26 different subclasses. NaPDoS2 analyses were run with default settings (e-value cutoff 1e-8 and 200aa minimum alignment length). KSα and KSβ sequences are grouped together.

Abbreviations: MetazoaFASI= type I FAS Metazoan-type; ProtistFASI= type I FAS Protist-type; FASII= type II FAS no subclass; Betabranch= type II beta-branching cassettes; Polyene= type II polyenes; Ape= type II aryl polyenes; Aro= type II aromatic unclassified; Angl= type II aromatic angucycline-derived I & II; Tetcyc= type II aromatic tetracycline-derived; Anth= type II aromatic anthracycline-derived I & II; Isochrom= type II aromatic isochromanequinone-derived; Tetcen= type II aromatic tetracenomycin-derived; Polyphen= type II aromatic pentangular polyphenol-derived; SPKS= type II aromatic spore pigment; *cis*-AT= type I modular *cis*-AT; cisloading= type I modular *cis*-AT loading module; cisHybridKS= type I modular *cis*-AT hybrid KS; cisOLS= type I modular *cis*-AT olefin synthase; cistandemECH= type I modular *cis*-AT tandem ECH; iPKS= type I iterative *cis*-AT no subclass; iPKSPUFA= type I iterative *cis*-AT PUFA (polyunsaturated fatty acids); Ened= type I iterative *cis*-AT enediynes; iPKSaromatic= type I iterative *cis*-AT aromatic; PTM= type I iterative *cis*-AT PTM-type (polycyclic tetramate macrolactam); HR= type I iterative *cis*-AT highly reducing; *trans*-AT= type I *trans*-AT no subclass; *trans*-hybridKS= type I *trans*-AT hybrid KS; *trans*-hybridKS0= type I *trans*-AT hybrid KS0 (non-elongating KS).

Table S9.

									Type II PKS											Type I PKS																
				FAS I								Aromatic									*cis*-AT modular					*cis*-AT iterative								*trans*-AT		
Metagenome	***# contigs***	***N50***	***Total NP2***	MetazoaFASI	ProtistFASI	FASII	MetazoaPKS	Betabranch	Polyene	Ape	Aro	Angl	Tetcyc	Anth	Isochrom	Tetcen	Polyphen	SPKS	*cis*-AT	cisloading	cisHybridKS	cisOLS	cistandemECH	iPKS	iPKSPUFA	Ened	iPKSaromatic	PTM	HR	*trans*-AT	*trans*-hybridKS	*trans*-hybridKS0				
MO18_007	152,894	5,918	145	-	-	61	-	-	5	3	1	5	-	-	-	-	-	-	19	3	5	1	-	1	34	-	-	-	-	7	-	-				
MO18_010	184,034	3,212	150	-	-	54	-	1	4	5	2	2	-	1	1	1	-	-	17	1	14	-	-	3	34	1	-	-	-	9	-	-				
MO18_039	336,958	1,769	58	-	-	13	4	-	1	1	-	1	-	-	1	-	-	-	8	-	17	-	-	-	3	1	-	-	-	8	-	-				
MO18_042	378,383	1,596	121	-	-	60	-	1	1	5	1	2	-	-	-	-	-	-	13	3	3	2	-	2	22	-	-	1	-	5	-	-				
MO18_071	37,150	2,093	87	-	1	23	-	-	-	2	-	1	-	-	-	-	1	-	24	-	9	3	-	-	5	-	-	1	-	16	-	1				
MO18_074	211,343	2,041	312	-	-	149	1	-	5	8	6	6	-	-	-	1	4	1	41	1	28	3	-	8	39	4	-	-	1	6	-	-				
MO18_103	186,468	1,851	269	-	1	114	-	1	6	13	3	1	-	-	1	1	6	-	34	3	15	3	1	4	46	3	-	-	-	13	-	-				
MO18_106	440,441	1,847	48	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-	19	1	-	6	-	-	7	-	-	-	-	3	-	-				
MO18_135	230,831	2,120	270	-	1	91	-	4	1	5	4	5	1	1	-	-	2	-	34	2	35	6	3	2	32	6	-	-	-	35	-	-				
MO18_138	104	1,300	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
MO18_167	311,846	2,402	706	-	-	272	-	7	5	21	12	13	-	4	1	1	8	1	83	11	48	18	3	8	129	18	2	1	-	39	1	-				
MO18_170	234,614	1,716	336	-	-	192	-	1	5	20	6	7	-	1	1	2	2	-	16	1	8	2	-	3	60	6	-	1	-	2	-	-				
MO18_180	182,647	1,599	227	-	-	125	-	1	5	12	-	4	-	-	1	-	3	-	13	1	10	4	1	5	29	3	-	-	-	10	-	-				
MO18_184	309,098	3,457	51	-	-	11	-	1	-	3	1	1	-	-	-	-	1	-	17	1	-	5	-	-	5	-	-	-	-	5	-	-				
MO18_188	259,773	2,959	505	-	-	188	1	5	5	9	4	10	1	-	-	-	7	-	106	4	41	20	3	7	53	8	1	1	-	30	1	-				
MO18_192	241,995	1,727	341	-	-	116	2	4	8	8	2	5	-	3	-	-	4	-	60	3	34	10	2	3	49	8	-	-	-	20	-	-				
MO18_199	229,239	2,042	200	3	-	77	-	4	2	3	1	2	-	1	1	-	2	-	46	3	2	1	4	3	31	2	-	-	-	12	-	-				
MO18_202	242,491	2,136	280	-	1	122	-	2	5	10	5	8	-	-	-	1	3	-	41	3	14	2	3	3	48	2	-	-	-	7	-	-				
MO18_231	110,677	2,357	91	-	-	30	-	1	-	5	-	2	-	-	-	-	1	-	22	1	2	-	1	-	20	1	-	-	-	5	-	-				
MO18_234	182,155	2,286	170	-	3	62	-	2	-	-	2	4	-	-	-	-	-	-	37	-	13	6	1	1	20	2	-	-	-	16	1	-				

Table S10. NaPDoS2 analysis of KS amplicon eSNaPD v2.0 data. NaPDoS2 detected and classified all 381 sequences from a New Mexico desert soil KS amplicon library (NM_KS_ARRY_LIB01, Owen *et al.* 2013 PNAS[22]). Additionally, NaPDoS2 classified all but one group of the 756 uncharacterized "Novel Clusters 1-60" from the same eSNaPD v2.0 soil amplicon library. NaPDoS2 settings: e-value cutoff 1e-8 and 50aa minimum alignment length (due to the amplicon sequence length). The "% ID" column lists the percent of sequences that were classified by NaPDoS2 (i.e. "Total NP2"/"# seq"; color scale of light grey (low % ID) to dark grey (high % ID)).

Abbreviations: *cis*-AT= type I modular *cis*-AT; cisloading= type I modular *cis*-AT loading module; cisHybridKS= type I modular *cis*-AT hybrid KS; cisOLS= type I modular *cis*-AT olefin synthase; cistandemECH= type I modular *cis*-AT tandem ECH; iPKSaromatic= type I iterative *cis*-AT aromatic; PTM= type I iterative *cis*-AT PTM-type (polycyclic tetramate macrolactam); NR= type I iterative *cis*-AT non-reducing; HR= type I iterative *cis*-AT highly reducing; *trans*-AT= type I *trans*-AT no subclass; *trans*-hybridKS= type I *trans*-AT hybrid KS.

Table S10.

				Type I PKS										
				cis-AT modular					*cis*-AT iterative				*trans*-AT	
% ID	**Sequence set**	**# seq**	*Total NP2*	*cis*-AT	cisloading	cisHybridKS	cisOLS	cistandemECH	iPKSaromatic	PTM	NR	HR	*trans*-AT	*trans*-hybridKS
102%	NM_KS_ARRY_LIB01	381	388	310	7	15	2	-	5	13	-	-	36	-
99%	NovClst1	192	191	2	-	-	-	-	-	-	189	-	-	-
100%	NovClst2	42	42	4	15	-	15	-	-	4	-	4	-	-
100%	NovClst3	38	38	3	-	-	-	-	-	35	-	-	-	-
94%	NovClst4	31	29	-	-	-	-	-	-	-	-	-	29	-
79%	NovClst5	29	23	-	-	22	-	-	-	-	-	-	-	1
108%	NovClst6	25	27	-	-	-	-	-	-	-	27	-	-	-
100%	NovClst7	22	22	-	-	22	-	-	-	-	-	-	-	-
112%	NovClst8	17	19	-	-	18	-	-	-	-	-	-	-	1
86%	NovClst9	14	12	-	-	11	-	-	-	-	-	-	-	1
92%	NovClst10	12	11	8	-	-	-	-	-	-	-	-	3	-
100%	NovClst11	12	12	6	-	-	-	-	-	-	-	-	6	-
100%	NovClst12	12	12	-	-	-	-	-	-	12	-	-	-	-
92%	NovClst13	12	11	-	-	11	-	-	-	-	-	-	-	-
110%	NovClst14	10	11	4	-	-	3	-	-	-	-	-	4	-
100%	NovClst15	10	10	-	-	-	10	-	-	-	-	-	-	-
100%	NovClst16	9	9	-	-	9	-	-	-	-	-	-	-	-
89%	NovClst17	9	8	7	-	-	1	-	-	-	-	-	-	-
111%	NovClst18	9	10	10	-	-	-	-	-	-	-	-	-	-
78%	NovClst19	9	7	5	-	-	-	1	-	-	-	-	1	-
89%	NovClst20	9	8	-	-	7	-	-	-	-	-	-	-	1
89%	NovClst21	9	8	8	-	-	-	-	-	-	-	-	-	-
100%	NovClst22	8	8	-	-	-	-	-	-	-	8	-	-	-
113%	NovClst23	8	9	9	-	-	-	-	-	-	-	-	-	-
113%	NovClst24	8	9	1	-	-	-	-	-	-	-	8	-	-
88%	NovClst25	8	7	-	-	-	-	-	-	-	-	-	7	-
100%	NovClst26	8	8	-	-	8	-	-	-	-	-	-	-	-
100%	NovClst27	8	8	8	-	-	-	-	-	-	-	-	-	-
114%	NovClst28	7	8	-	-	-	-	-	-	-	8	-	-	-
86%	NovClst29	7	6	-	-	6	-	-	-	-	-	-	-	-
100%	NovClst30	7	7	-	-	7	-	-	-	-	-	-	-	-
100%	NovClst31	7	7	4	-	-	3	-	-	-	-	-	-	-
100%	NovClst32	7	7	-	-	7	-	-	-	-	-	-	-	-
86%	NovClst33	7	6	-	-	-	-	-	-	-	-	-	6	-
100%	NovClst34	7	7	-	-	7	-	-	-	-	-	-	-	-
100%	NovClst35	6	6	1	1	-	-	-	-	-	-	-	4	-
67%	NovClst36	6	4	-	-	-	-	-	-	-	-	-	4	-
100%	NovClst37	6	6	-	-	6	-	-	-	-	-	-	-	-
83%	NovClst38	6	5	-	-	5	-	-	-	-	-	-	-	-
100%	NovClst39	6	6	6	-	-	-	-	-	-	-	-	-	-
67%	NovClst40	6	4	-	-	4	-	-	-	-	-	-	-	-
100%	NovClst41	6	6	6	-	-	-	-	-	-	-	-	-	-
0%	NovClst42	6	0	-	-	-	-	-	-	-	-	-	-	-
100%	NovClst43	6	6	1	-	-	5	-	-	-	-	-	-	-
100%	NovClst44	5	5	-	-	-	-	-	-	-	-	-	5	-
100%	NovClst45	5	5	-	-	5	-	-	-	-	-	-	-	-
100%	NovClst46	5	5	5	-	-	-	-	-	-	-	-	-	-
140%	NovClst47	5	7	2	-	-	4	-	-	-	-	1	-	-
60%	NovClst48	5	3	-	-	-	-	-	-	-	-	-	3	-
100%	NovClst49	4	4	-	-	4	-	-	-	-	-	-	-	-
100%	NovClst50	4	4	-	-	4	-	-	-	-	-	-	-	-
100%	NovClst51	4	4	-	-	4	-	-	-	-	-	-	-	-
100%	NovClst52	4	4	-	-	-	-	-	-	-	-	-	4	-
25%	NovClst53	4	1	1	-	-	-	-	-	-	-	-	-	-
100%	NovClst54	4	4	4	-	-	-	-	-	-	-	-	-	-
100%	NovClst55	4	4	-	-	4	-	-	-	-	-	-	-	-
100%	NovClst56	4	4	-	-	-	-	-	-	-	-	-	4	-
100%	NovClst57	4	4	-	-	4	-	-	-	-	-	-	-	-
75%	NovClst58	4	3	3	-	-	-	-	-	-	-	-	-	-
75%	NovClst59	4	3	-	-	3	-	-	-	-	-	-	-	-
175%	NovClst60	4	7	-	-	-	-	-	-	-	7	-	-	-

Table S11. **NaPDoS2 analysis of 12 type II KS amplicon datasets from Borsetto *et al.* 2019[23].** The total number of amplicon sequences not detected by NaPDoS2 represented 36-95% of the sequences in the amplicon libraries ("# seqs"). Of the KS sequences that were detected by NaPDoS2, 19-93% were classified as type II PKSs and could be assigned to a wide range of type II subclasses while the remainder were classified as type II fatty acid synthases (FASII). NaPDoS2 settings: e-value cutoff 1e-8 and 50aa minimum alignment length (due to the amplicon sequence length).

Abbreviations: FASII= type II FAS no subclass; Betabranch= type II beta-branching cassettes; Polyene-KSα= type II polyenes KSα; Ape-KSα= type II aryl polyenes KSα; Aro-KSα= type II aromatic unclassified KSα; Angl-I-KSα= type II aromatic angucycline-derived I KSα; Angl-II-KSα= type II aromatic angucycline-derived II KSα; Tetcyc-KSα= type II aromatic tetracycline-derived KSα; Anth-I-KSα= type II aromatic anthracycline-derived I KSα; Anth-I-KSβ= type II aromatic anthracycline-derived I KSβ; Anth-II-KSα= type II aromatic anthracycline-derived II KSα; Isochrom-KSα= type II aromatic isochromanequinone-derived KSα; Tetcen-KSα= type II aromatic tetracenomycin-derived KSα; Tetcen-KSβ= type II aromatic tetracenomycin-derived KSβ; Polyphen-KSα= type II aromatic pentangular polyphenol-derived KSα; Polyphen-KSβ= type II aromatic pentangular polyphenol-derived KSβ; SPKS-KSα= type II aromatic spore pigment KSα; *cis*-AT= type I modular *cis*-AT; cisHybridKS= type I modular *cis*-AT hybrid KS.

Table S11.

				Type II PKS																Type I PKS	
							Aromatic													*cis*-AT modular	
Dataset	**# seqs**	***Total NP2***	FASII	Betabranch	Polyene-KSα	Ape-KSα	Aro-KSα	Angl-I-KSα	Angl-II-KSα	Tetcyc-KSα	Anth-I-KSα	Anth-I-KSβ	Anth-II-KSα	Isochrom-KSα	Tetcen-KSα	Tetcen-KSβ	Polyphen-KSα	Polyphen-KSβ	SPKS-KSα	*cis*-AT	cisHybridKS
S31_Antarctica	34,600	12,757	9,593	6	54	2	63	419	163	13	10	1	21	201	75	3	333	20	1,780	-	-
S32_Antarctica	31,118	7,905	6,414	4	50	12	52	369	51	13	42	1	11	134	47	1	343	16	344	1	-
S33_Antarctica	14,703	774	328	-	8	-	18	264	1	1	20	-	-	31	30	2	18	9	44	-	-
S22_AlgeriaB3	166,814	9,345	1,172	1	177	15	1,253	1,003	37	4	2,573	-	76	366	461	-	1,370	-	836	1	-
S23_AlgeriaB3	119,567	19,317	3,155	4	429	38	836	4,648	22	39	4,687	-	106	418	954	-	1,212	-	2,768	1	-
S24_AlgeriaB3	126,771	21,643	3,072	-	373	26	1,354	4,149	27	4	6,824	-	148	414	1,075	-	2,097	-	2,080	-	-
S28_CubaFir	182,418	13,220	3,605	-	308	17	931	2,718	9	10	1,174	-	90	851	48	-	527	-	2,931	1	-
S29_CubaFir	194,450	18,079	2,394	-	146	12	1,279	7,113	8	3	3,331	-	18	1,174	18	-	896	-	1,687	-	-
S30_CubaFir	204,614	30,393	2,701	-	1,056	11	993	7,993	6	8	1,822	-	194	4,820	39	-	973	-	9,768	9	-
S37_Warwick	241,475	115,324	19,560	8	560	30	3,964	15,941	160	20	6,114	-	269	38,472	38	-	8,868	17	21,276	23	4
S38_Warwick	110,892	58,876	7,728	4	321	22	4,390	6,556	124	1	3,158	-	269	7,026	38	-	4,913	11	24,312	1	2
S39_Warwick	135,287	86,200	5,716	1	118	10	2,760	12,604	344	6	2,936	-	600	33,933	60	-	4,122	15	22,974	-	1

Table S12. **NaPDoS2 analysis of 5,000 randomly selected KS amplicon sequences from the Elfeki *et al.* 2018[15] "Chitin" type I** (SRR7206837_H054B_Chitin_KSdomain) **and type II** (SRR7206805_H054B_Chitin_KSalphadomain) **KS datasets run at varying minimum amino acid alignment lengths.** Decreasing the minimum amino acid alignment length increases the number of KSs detected but also increases the likelihood of false positives and misclassifications, as may be evidenced by the detection of type I KSs in the type II dataset. Notably, below an alignment length of 100aa, the number of KSs detected in the type I dataset exceeds the number of sequences analyzed, as can be expected when shorter domain fragment hits are identified from the same longer amplicon sequences.

Table S12.

Type II KSα: Chitin				
Alignment Length	Class	Subclass	NP2 Hits	*Total NP2 hits*
200aa	**-**	-	0	0
150aa	type II aromatic	pentangularpolyphenolKSa	1	1
100aa	type II aromatic	angucycline I KSa	469	1,229
	type II aromatic	isochromanequinone KSa	225	
	type II aromatic	pentangular polyphenol KSa	200	
	type II aromatic	unclassified KSa	146	
	type II aromatic	tetracenomycin KSa	111	
	type II aromatic	anthracycline I KSa	70	
	type I modular cis-AT	no subclass	2	
	type II aromatic	angucycline II KSa	2	
	type II aromatic	spore pigment KSa	1	
	type I trans-AT	hybrid KS	1	
	type I trans-AT	no subclass	1	
	type II aromatic	anthracycline II KSa	1	
50aa	type II aromatic	angucycline I KSa	958	2,999
	type II aromatic	pentangular polyphenol KSa	673	
	type II aromatic	isochromanequinone KSa	570	
	type II aromatic	unclassified KSa	378	
	type II aromatic	tetracenomycin KSa	221	
	type II aromatic	anthracycline I KSa	132	
	type II aromatic	spore pigment KSa	31	
	type I modular cis-AT	no subclass	20	
	type II aromatic	anthracycline II KSa	4	
	type II aromatic	tetracycline KSa	4	
	type II aromatic	angucycline II KSa	4	
	type I modular cis-AT	hybrid KS	2	
	type I trans-AT	hybrid KS	1	
	type I trans-AT	no subclass	1	
30aa	type II aromatic	angucycline I KSa	994	3,099
	type II aromatic	pentangular polyphenol KSa	693	
	type II aromatic	isochromanequinone KSa	584	
	type II aromatic	unclassified KSa	381	
	type II aromatic	tetracenomycin KSa	224	
	type II aromatic	anthracycline I KSa	138	
	type II aromatic	spore pigment KSa	40	
	type I modular cis-AT	no subclass	22	
	type II aromatic	tetracycline KSa	9	
	type II aromatic	anthracycline II KSa	5	
	type II aromatic	angucycline II KSa	5	
	type I modular cis-AT	hybrid KS	2	
	type I trans-AT	hybrid KS	1	
	type I trans-AT	no subclass	1	

Type I KS: Chitin				
Alignment Length	Class	Subclass	NP2 Hits	*Total NP2 hits*
200aa	type I modular cis-AT	no subclass	21	23
	type I iterative cis-AT	aromatic	2	
150aa	type I modular cis-AT	no subclass	385	423
	type I iterative cis-AT	aromatic	24	
	type I trans-AT	no subclass	8	
	type I trans-AT	hybrid KS	6	
100aa	type I modular cis-AT	no subclass	3,406	3,594
	type I trans-AT	no subclass	75	
	type I iterative cis-AT	aromatic	57	
	type I modular cis-AT	hybrid KS	39	
	type I trans-AT	hybrid KS	15	
	type II aromatic	pentangular polyphenol KSa	1	
	type II aromatic	spore pigment KSa	1	
50aa	type I modular cis-AT	no subclass	7,441	7,768
	type I trans-AT	no subclass	139	
	type I modular cis-AT	hybrid KS	85	
	type I iterative cis-AT	aromatic	72	
	type I trans-AT	hybrid KS	17	
	type II aromatic	pentangular polyphenol KSa	5	
	type II aromatic	angucycline I KSa	3	
	type II aromatic	spore pigment KSa	2	
	type II aromatic	anthracycline I KSa	1	
	type II aromatic	isochromanequinone KSa	1	
	type II aromatic	tetracenomycin KSa	1	
	type II aromatic	unclassified KSa	1	
30aa	type I modular cis-AT	no subclass	7,600	7,930
	type I trans-AT	no subclass	140	
	type I modular cis-AT	hybrid KS	86	
	type I iterative cis-AT	aromatic	72	
	type I trans-AT	hybrid KS	17	
	type II aromatic	pentangular polyphenol KSa	5	
	type II aromatic	angucycline I KSa	3	
	type II aromatic	spore pigment KSa	3	
	type II aromatic	anthracycline I KSa	1	
	type II aromatic	isochromanequinone KSa	1	
	type II aromatic	tetracenomycin KSa	1	
	type II aromatic	unclassified KSa	1	

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