Comparative Genomics and Pan-Genomics of the Myxococcaceae, including a Description of Five Novel Species: *Myxococcus eversor* sp. nov., *Myxococcus llanfairpwllgwyngyllgogerychwyrndrobwllllantysiliogogogochensis* sp. nov., *Myxococcus vastator* sp. nov., *Pyxidicoccus caerfyrddinensis* sp. nov., and *Pyxidicoccus trucidator* sp. nov.

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Abstract

Members of the predatory Myxococcales (myxobacteria) possess large genomes, undergo multicellular development, and produce diverse secondary metabolites, which are being actively prospected for novel drug discovery. To direct such efforts, it is important to understand the relationships between myxobacterial ecology, evolution, taxonomy, and genomic variation.

This study investigated the genomes and pan-genomes of organisms within the Myxococcaceae, including the genera *Myxococcus* and *Corallococcus*, the most abundant myxobacteria isolated from soils. Previously, ten species of *Corallococcus* were known, whereas six species of *Myxococcus* phylogenetically surrounded a third genus (*Pyxidicoccus*) composed of a single species. Here, we describe draft genome sequences of five novel species within the Myxococcaceae (*Myxococcus eversor*, *Myxococcus llanfairpwllgwyngyllgogerychwyrndrobwllllantysiliogogogochensis*, *Myxococcus vastator*, *Pyxidicoccus caerfyrddinensis*, and *Pyxidicoccus trucidator*) and for the *Pyxidicoccus* type species strain, *Pyxidicoccus fallax* DSM 14698[T]. Genomic and physiological comparisons demonstrated clear differences between the five novel species and every other *Myxococcus* or *Pyxidicoccus* spp. type strain.

Subsequent analyses of type strain genomes showed that both the *Corallococcus* pan-genome and the combined *Myxococcus* and *Pyxidicoccus* (*Myxococcus*/*Pyxidicoccus*) pan-genome are large and open, but with clear differences. Genomes of *Corallococcus* spp. are generally smaller than those of *Myxococcus*/*Pyxidicoccus* spp. but have core genomes three times larger. *Myxococcus*/*Pyxidicoccus* spp. genomes are more variable in size, with larger and more unique sets of accessory genes than those of *Corallococcus* species. In both genera, biosynthetic gene clusters are relatively enriched in the shell pan-genomes, implying they grant a greater evolutionary benefit than other shell genes, presumably by conferring selective advantages during predation.

Key words: antimicrobials, pharmaceuticals, comparative genomics, myxobacteria, predator, prey.

Significance

When comparing the genomes of organisms it is important to take into account the relatedness of those organisms, but this is often precluded because there is inadequate understanding of the studied organisms' classification. Here, we characterized five novel species of prokaryotes belonging to the myxobacteria, allowing a consistent comparison of the pan-genomes of two myxobacterial genera. We discovered that the two genera exhibit markedly different patterns of genomic diversity, with members of the *Myxococcus*/*Pyxidicoccus* genus exhibiting larger and more diverse genomes than members of the *Corallococcus* genus.

Introduction

The search for novel antimicrobial and other therapeutic bioactive molecules is an ongoing endeavor, and soil-dwelling microbes remain attractive targets for bioprospecting efforts. Within the soil microbiome, predatory microbes are known to be a particularly good source of bioactive compounds, as might be expected given their antimicrobial lifestyle. For instance, the predatory myxobacteria (Order Myxococcales) are the origin of numerous bioactive compounds, including antibiotics, antivirals, and cytotoxins (Herrmann et al. 2017; Mulwa and Stadler 2018). Sophisticated hunters, myxobacteria are able to sense signals from prey, which stimulates predatory behaviors and suppresses multicellular fruiting body development (Lloyd and Whitworth 2017). Myxobacterial predation has historically been described as wolf-pack predation (Pérez et al. 2016; Livingstone, Millard, et al. 2018). This reflects the mechanism of predation, which involves constitutive secretion of toxic compounds into the public commons, although whether this makes predation cooperative is debatable (Marshall and Whitworth 2019; Furness et al. 2020). It has also become apparent recently, that myxobacteria can be readily isolated from marine sources, not just from soils (Gemperlein et al. 2018). Marine myxobacteria isolates are often novel taxonomically and produce diverse and unusual bioactive molecules (Amiri Moghaddam et al. 2018; Garcia et al. 2018). Such discoveries have led to exploration of the relationships of myxobacterial evolution, classification, and ecology, with their production of bioactive compounds (Hoffmann et al. 2018).

The Myxococcaceae are the family of myxobacteria most commonly isolated from soils (Mohr et al. 2016; Livingstone et al. 2017). At the time of the seminal phylogenetic study of Garcia et al. (2010), eight species within three genera were recognized within the Myxococcaceae (*Corallococcus coralloides*, *Corallococcus exiguus*, *Myxococcus fulvus*, *Myxococcus macrosporus*, *Myxococcus stipitatus*, *Myxococcus virescens*, *Myxococcus xanthus*, and *Pyxidicoccus fallax*). Since then, several further species and genera have been identified and assigned to the Myxococcaceae. As of 2020, there are five genera and 20 species described: *Aggregicoccus edonensis* (Sood et al. 2015), *Corallococcus* (ten species, see Livingstone et al. 2020), *Myxococcus* (seven species, Garcia et al. 2010; Sharma et al. 2016), *P. fallax*, and *Simulacricoccus ruber* (Garcia and Müller 2018). 16S rRNA gene phylogenetic analysis shows that the genus *Myxococcus* is split into two groups of species, one of which (containing *M. stipitatus* and *M. fulvus*) bifurcates with *P. fallax* (Garcia et al. 2010). In this study, we have therefore considered *Myxococcus* and *Pyxidicoccus* to be a single genus, referring to it hereafter as the *Myxococcus*/*Pyxidicoccus* genus.

Traditional myxobacterial classification has relied on polyphasic phenotypic characterization, however, deducing the genome sequences of isolates allows their taxonomic assignment on the basis of conserved gene sequence similarity. For instance, the average nucleotide identity (ANI) is a metric that can be used to compare strains, with members of the same species typically giving ANI values above 95%. Similarly, digital DNA–DNA hybridization (dDDH) values above 70% suggest that two strains belong to the same species (Chun et al. 2018). Through ANI and dDDH comparisons, genome sequencing allows the rapid taxonomic assignment of new strains, with greater resolution than 16S rRNA gene phylogenetics (Richter and Rosselló-Móra 2009).

The availability of genome sequences also allows investigations into gene functions and organismal evolution. For instance, the presence/absence of genes can be correlated with the phenotypic properties of strains through approaches such as genome-wide association studies (GWASs), to identify potentially causal relationships. For example, comparing the predatory activity of myxobacterial isolates with the presence/absence of individual genes through GWAS has allowed the identification of predation-related genes (Sutton et al. 2019).

Comparative genomics analyses of 23 *Corallococcus* spp. strains, and ten *M. xanthus* strains, have revealed that myxobacterial pan-genomes are open, with large accessory pan-genomes (Livingstone, Morphew, Whitworth 2018; Zwarycz et al. 2020). The majority of biosynthetic gene clusters (BGCs) responsible for production of secondary metabolites were found to lie within the accessory pan-genome, conferring considerable metabolic individuality on species and strains within the family (Livingstone, Morphew, Whitworth 2018). However, previous pan-genomic and GWAS analyses have been limited by the lack of available genome sequences, necessitating using genomes from organisms of uncertain

taxonomic relatedness (Livingstone, Morphew, Whitworth 2018; Sutton et al. 2019). To understand myxobacterial genome biology better, it is important to further explore the genomic and phenotypic diversity of natural isolates, while defining their taxonomy rigorously.

Toward that end, in this study, five isolates representing candidate novel species were characterized using both genomic and experimental approaches, integrating phenotypic, biochemical, and genomic analyses. For comparison, all available Myxococcaceae species-type strains were also characterized (except *A. edonensis*, *Myxococcus hansupus*, and *S. ruber*, which were not available through culture collections, but for which sequence data were available). All five candidate strains were confirmed as belonging to novel species, bringing the number of known species in each of the *Corallococcus* and *Myxococcus*/*Pyxidicoccus* genera above ten. This then allowed us to undertake a rigorous comparison of the pan-genomes of the *Corallococcus* and *Myxococcus*/*Pyxidicoccus* genera, using the type strains from each constituent species.

Materials and Methods

Isolation, Growth Conditions, and Identification

Strains were isolated using an experimental strategy that selected for motile bacteriolytic strains that form fruiting bodies, as described previously (Livingstone et al. 2017), using *Escherichia coli* ATCC 25922 cells as bait. Isolates were cultivated on VY-2 medium (0.5% w/v dried baker's yeast, 0.1% w/v $CaCl_2{\cdot}2H_2O$, and 1.5% w/v agar). An initial identification of isolates using EzBioCloud (Yoon et al. 2017) was undertaken by sequencing polymerase chain reaction-amplified portions of the 16S rRNA gene, using primers F27 and R1389, as described previously (Livingstone et al. 2017). Type strains were obtained from the DSMZ, Germany (*M. fulvus* DSM 16525^T, *M. macrosporus* DSM 14697^T, *M. stipitatus* DSM 14675^T, *M. virescens* DSM 2260^T, *M. xanthus* DSM 16526^T, and *P. fallax* DSM 14698^T).

Genome Sequencing and Comparative Genomics

Draft genome sequences of isolates and *P. fallax* DSM 14698^T were obtained either from MicrobesNG (Birmingham, UK) or from the Centre for Genomic Research (Liverpool, UK). Submitted samples were streaked onto a variety of media to check the samples were free of contaminating organisms. The sequencing platform used by both centers was the Illumina Hiseq 2500, and paired-end reads were quality controlled with BWA-MEM, mapped by Kraken 2.0, and assembled by SPAdes 3.7 (Li and Durbin 2009; Bankevich et al. 2012; Wood and Salzberg 2014). The taxonomic distribution of reads was mapped using Kraken 2.0 and <0.01% of reads mapped to nonmyxobacterial genomes. Assembled sequences were deposited in GenBank (excluding contigs <800 bp long, and with <15-fold coverage), and annotation of coding sequences and other features was provided by PGAP-4 (Haft et al. 2018).

Previously sequenced genomes of other type strains were obtained from GenBank. Complete 16S rRNA gene sequences were extracted from genome sequences (a single complete 16S rRNA gene sequence was present in each assembly) and phylogenetic analysis undertaken (Neighbor-Joining trees of ClustalW alignments, with 1,000 bootstraps), using Phylogeny.fr (Dereeper et al. 2008). ANI comparisons and resulting distance trees were produced using the ANI–matrix genome distance calculator (Rodriguez and Konstantinidis 2016). dDDH values were obtained from the GGDC server, using recommended formula 2, which works best for draft genomes (Meier-Kolthoff et al. 2013). GBDP (Genome BLAST Distance Phylogeny) trees were obtained from the TYGS server (Meier-Kolthoff and Göker 2019), using default formula d_4. The antiSMASH 5.0 server was queried to categorize BGCs (Blin et al. 2019). For pan-genome analysis .gff files were generated for each genome using Prokka (Seemann 2014) and analyzed using Roary set to a 90% BlastP cut-off (Page et al. 2015). Roary divides proteins from all genomes into clusters of orthologs and then assesses how many genomes include a member of each cluster. Genes were classified as core (present in every genome), or accessory (non-core genes). Accessory genes were then subcategorized as "cloud" (found in a single genome), or "shell" (found in more than one, but not all, genomes). Roary output graphs were visualized using the recommended R scripts.

Physiology, Biochemical Activities, and Predation Assays

Predatory activity was quantified using lawn-based assays where suspensions of predator cells were spotted onto lawns of model prey organisms, as described previously (Livingstone et al. 2017). The prey organisms used in this study were *E. coli* TOP10 (a Gram-negative bacterium), *Clavibacter nebraskensis* DSM 7483 (a Gram-positive bacterium), and *Ustilago maydis* DSM 14603 (a fungus). Predatory activity was assessed after 7 days' incubation at 30 °C, by measuring the diameter of the zones of prey killing and myxobacterial growth (in triplicate). To assess ability to grow at different temperatures, pH values, and salinities, colony expansion of predator strains was assessed by visual scoring of inoculated VY-2 plates (amended with KOH/HCl or NaCl as required, in triplicate). Scanning electron micrographs were generated using the same sample preparation and imaging regimes as described previously (Livingstone, Morphew, Cookson, et al. 2018). The BioMérieux API 20 NE kit allowed assessment of biochemical activities and was used according to the manufacturer's instructions.

Table 1
Strains Isolated and/or Genome Sequenced as Part of This Study

Strain	Genome Accession	Source of Soil Sample	Gridref	Closest-Type Strain (16S)	Genome Size (Mb)	%GC	Contigs	L50	N50
AB053B	JAAIXY01	Aberystwyth	52.41°N 4.08°W	*M. macrosporus* DSM 14697^T	11.394	68.9	135	12	313,084
AM301	JAAIYB01	Anglesey	53.22°N 4.19°W	*M. xanthus* DSM 16526^T	8.99	69.9	1,008	172	15,871
AM401	VIFM01	Anglesey	53.22°N 4.19°W	*M. macrosporus* DSM 14697^T	12.41	68.7	1,077	102	35,723
CA032A	JAAIYA01	Carmarthen	51.86°N 4.31°W	*P. fallax* DSM 14698^T	13.43	70.2	202	12	321,034
CA060A	JAAIXZ01	Llansteffan	51.77°N 4.39°W	*P. fallax* DSM 14698^T	12.65	70.3	153	10	375,293
P. fallax DSM 14698^T	JABBJJ01	—	—	—	13.53	70.5	825	132	31,399

NOTE.—The sites from which soil samples yielding isolates were collected are indicated. The type strains with the most similar 16S rRNA gene sequence are also provided. L50 and N50 values indicate that the L50th largest contigs together constitute half the genome. The L50th largest contig has a size of N50 bp. For example, the largest 12 contigs of the AB053B genome together comprise more than 50% of the genome sequence, and the 12th contig is 313,084 bp long.

Results

16S rRNA Gene and Draft Genome Sequences Indicate Five Novel Myxococcaceae Species

Five previously isolated bacteriolytic swarming myxobacterial strains were selected for further study, which were candidates for being new species within *Myxococcus/Pyxidicoccus* based on analysis of their 16S rRNA gene sequences. Isolation of three of the strains (AB053B, CA032A, and CA060A) has been described previously (Livingstone et al. 2017), whereas the remaining two strains (AM301 and AM401) were isolated from soil samples taken on the island of Anglesey, UK. General features of the five isolates are provided in table 1, including their site of origin and their most similar type strains as determined by 16S rRNA gene sequence analysis.

A phylogenetic tree of 16S rRNA gene sequences is shown in figure 1*A*, for the five isolates, and for each type strain within the Myxococcaceae (16S gene sequences of type strains were obtained from GenBank). The tree shows clustering of type strains according to genus, with *S. ruber* and *A. edonensis* particularly distinct from the other genera. Two isolates group with *P. fallax* (CA032A and CA060A), whereas the other three (AB053B, AM301, and AM401) group with *Myxococcus* spp. type strains. The same pattern of clustering was also seen with maximum likelihood trees of single copy housekeeping genes/proteins including, for instance, the enzyme acetylglutamate kinase and the noncoding RNA RnpB (data not shown).

Draft genome sequences were obtained for each isolate (and for the previously unsequenced *P. fallax*-type strain DSM 14698^T) and their summary statistics, which are typical for Myxococcaceae, are shown in table 1, albeit with *Pyxidicoccus* spp. exhibiting slightly higher %GC content and larger genome sizes than *Myxococcus* spp. ANI and dDDH values were determined for all combinations of isolates and Myxococcaceae-type strains. A matrix of dDDH values and ANI values is provided in table 2, and phylogenetic trees based on dDDH and ANI values are shown in figure 1*B* and *C*, respectively. The dDDH and ANI trees show the same groupings as the 16S tree, with CA032A and CA060A grouping with *P. fallax*, and AB053B, AM301, and AM401 grouping with the *Myxococcus* spp. type strains.

Strains with ANI values below 95% and DDH values below 70% are considered to be members of different species (Chun et al. 2018). All comparisons gave dDDH values below 70% and ANI values below 95% (except the *M. xanthus* vs. *M. virescens* comparison), therefore we propose that CA032A and CA060A belong to novel species within the *Pyxidicoccus* genus (*Pyxidicoccus caerfyrddinensis* and *Pyxidicoccus trucidator*, respectively), whereas AB053B, AM301, and AM401 represent novel species within the *Myxococcus* genus (*Myxococcus eversor*, *Myxococcus vastator*, and *Myxococcus llanfairpwllgwyngyllgogerychwyrndrobwllllantysiliogogogochensis*, respectively).

Physiological Assays of Candidate Strains Support the Proposal of Five Novel Species

Cells of strains AB053A, AM301, AM401, CA032A, and CA060A were imaged by scanning electron microscopy. Typical examples are presented in figure 2 and show rod-shaped cells of the dimensions typical for Myxococcaceae (0.4–0.8 μm wide by 3.0–8.0 μm long). Colonies of all strains were motile on solid VY-2 medium, and their growth characteristics were determined at various pH values, temperatures, and salinities (table 3). AB053B, AM401, and CA032A were particularly adaptable, being able to grow well under all conditions tested. AM301 and CA060A were more particular in their growth requirements, only growing significantly at higher pH values, lower temperatures, and low salinity (table 3).

The biochemical activities of candidate strains were tested using API kits and the results are shown in table 4A. CA032A and CA060A gave similar profiles of activity to each other and to *P. fallax* DSM 14698^T. The activity profile of AM301 was most similar to that of *M. macrosporus* DSM 14697^T, AM401

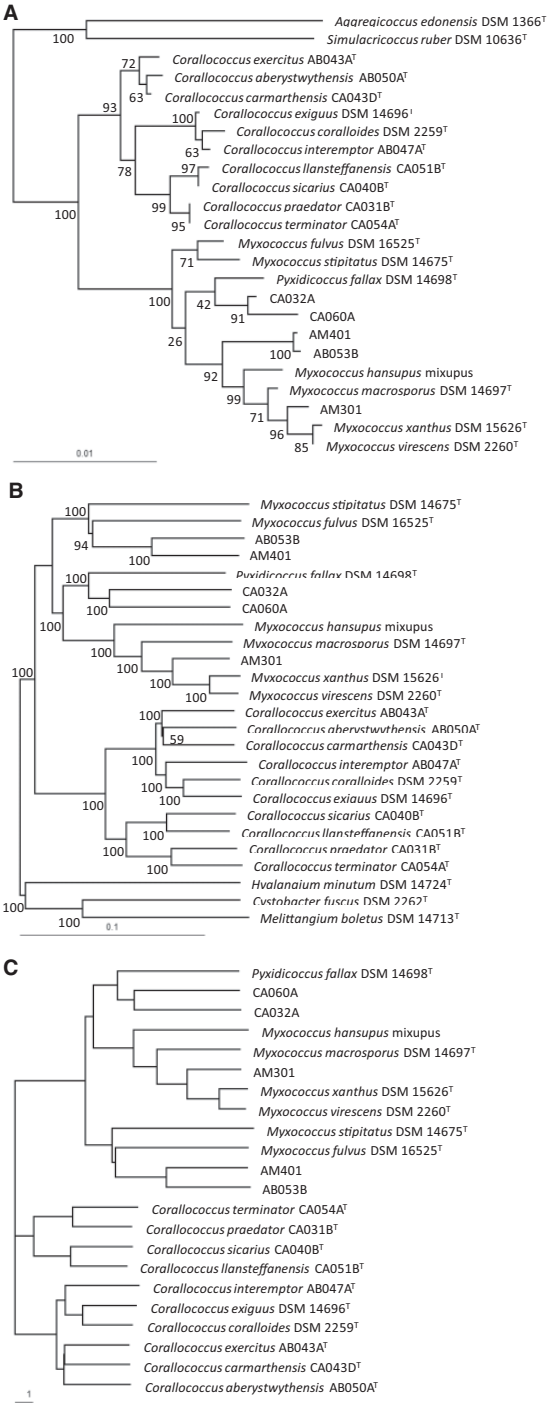

Fig. 1.—Dendrograms of isolates and myxobacterial type strains. (*A*) Neighbour-joining 16S rRNA sequence phylogenetic tree, (*B*) Genome BLAST Distance Phylogeny (GBDP) tree derived from dDDH values, (*C*) clustering tree based on ANI values. Support values are shown as a percentage in trees A and B (1,000 bootstraps). Bars show substitution frequency (*A*) and GBDP distances (*B*). *Hyalangium minutum*, *Cystobacter fuscus*, and *Melittangium boletus* are included in the GBDP tree as an outgroup.

was most similar to *M. virescens*, whereas that of AB053B was similar to those of the *M. fulvus* and *M. stipitatus*-type strains—relationships which largely (except for AM401) reflect the genetic/genomic relatedness of strains seen in figure 1. The predatory activity of each strain was also assessed using a lawn-based assay wherein suspensions of predatory cells were spotted onto lawns of prey, and the size of the resulting "zone of predation" measured after 7 days. Table 4B indicates above-average and below-average predatory activity against three different prey: *E. coli* TOP10 (Gram-negative bacterium), *Cl. nebraskensis* DSM 7483 (Gram-positive bacterium), and *U. maydis* DSM 14603 (fungus). *Myxococcus fulvus* and *M. stipitatus* were particularly good predators, with above-average activity against all three prey.

The *Myxococcus*/*Pyxidicoccus* Pan-Genome Is Larger and More Diverse than That of *Corallococcus*

On the basis of ANI and dDDH comparisons, it would appear that *M. xanthus* and *M. virescens* represent a single species. We have therefore combined the two species as *M. virescens*/*xanthus* for the rest of this study and use the *M. virescens* DSM 2260 genome sequence as the type genome, as *M. virescens* has priority over *M. xanthus* (Thaxter 1892 vs. Beebe 1941, respectively). The dDDH and ANI values also show that *M. stipitatus* and *M. fulvus* are as similar to *Pyxidicoccus* spp. as they are to the other *Myxococcus* spp. (table 2), supporting our treatment of the *Myxococcus* and *Pyxidicoccus* genera as a single *Myxococcus*/*Pyxidicoccus* genus. With the identification here of five new *Myxococcus*/*Pyxidicoccus* spp., there are now similar numbers of *Corallococcus* spp. and *Myxococcus*/*Pyxidicoccus* spp. (10 and 11, respectively).

The pan-genomes of *Corallococcus* spp. and *Myxococcus*/*Pyxidicoccus* spp. were characterized using the Roary algorithm, queried with the type strain genome sequence for each species. The resulting pan-genome metrics are provided in table 5, including a breakdown of each pan-genome into core genes (those found in every genome), and accessory genes (non-core genes), which were then subdivided into "cloud" genes (those present in a single genome), and "shell" genes (genes found in more than one, but not all, genomes). Supplementary file 1, Supplementary Material online, provides the orthologous clusters identified by Roary and also indicates which genomes contained an ortholog from each cluster. Plots of core genome and pan-genome sizes, as a function of the number of included genomes, are illustrated for both *Corallococcus* spp. and *Myxococcus*/*Pyxidicoccus* spp. in figure 3, whereas the distribution of genes across genomes is shown in figure 4. As further genomes are included, both genera exhibit a rapid decline in the size the core genome, from around 8,200 to 2,400 genes for *Corallococcus* spp., and from around 8,800 to

Table 2

ANI and dDDH Values for Pairwise Comparisons between Isolates, *Myxococcus* spp.-Type Strains, *Pyxidicoccus fallax* DSM 14698^T, and *Corallococcus coralloides* DSM 2259^T

	CA032A	CA060A	Pf	Mv	Mx	AM301	Mm	Mh	AB053B	AM401	Mf	Ms	Cc
CA032A	100	88.2	86.2	83.2	83.1	83.6	83.5	82.9	82.2	82.3	82.2	81.8	80.8
CA060A	33.1	100	86.5	83.4	83.4	83.8	83.8	83.1	82.5	82.5	82.2	81.9	80.7
Pf	29.6	30.0	100	83.3	83.2	83.7	83.8	83.0	82.4	82.4	82.5	82.1	80.9
Mv	24.7	25.0	25.1	100	97.0	93.7	90.4	87.3	81.8	82.2	81.8	81.5	80.5
Mx	24.6	24.8	25.0	73.1	100	93.6	90.2	87.2	81.6	82.1	81.7	81.4	80.4
AM301	25.4	25.6	25.7	52.8	52.7	100	90.8	87.7	82.0	82.3	82.2	82.0	80.8
Mm	25.0	25.3	25.5	40.5	40.1	41.6	100	87.6	82.0	82.5	82.2	81.9	80.8
Mh	24.1	24.5	24.6	32.0	23.0	32.9	32.8	100	81.6	81.7	81.7	81.5	80.5
AB053B	23.4	23.6	23.7	22.8	22.7	23.5	23.1	22.7	100	90.8	85.1	84.6	80.2
AM401	23.7	29.9	23.9	23.6	23.6	23.8	23.9	23.0	41.4	100	85.2	84.8	80.4
Mf	23.3	23.4	23.7	22.9	22.7	23.5	23.3	22.7	28.0	28.4	100	84.3	80.4
Ms	22.8	22.9	23.2	22.5	27.6	23.0	22.8	22.2	27.1	27.6	26.5	100	80.2
Cc	21.4	21.6	21.9	21.3	21.3	21.9	21.5	21.2	21.0	21.3	21.2	20.9	100

NOTE.—dDDH values are shown above the diagonal and ANI values are shown above the diagonal. ANI values of 90% or more, and dDDH values above 40% are shaded gray. Pf, *P. fallax*; Mv, *M. virescens*; Mx, *M. xanthus*; Mm, *M. macrosporus*; Mf, *M. fulvus*; Ms, *M. stipitatus*; Cc, *C. coralloides*.

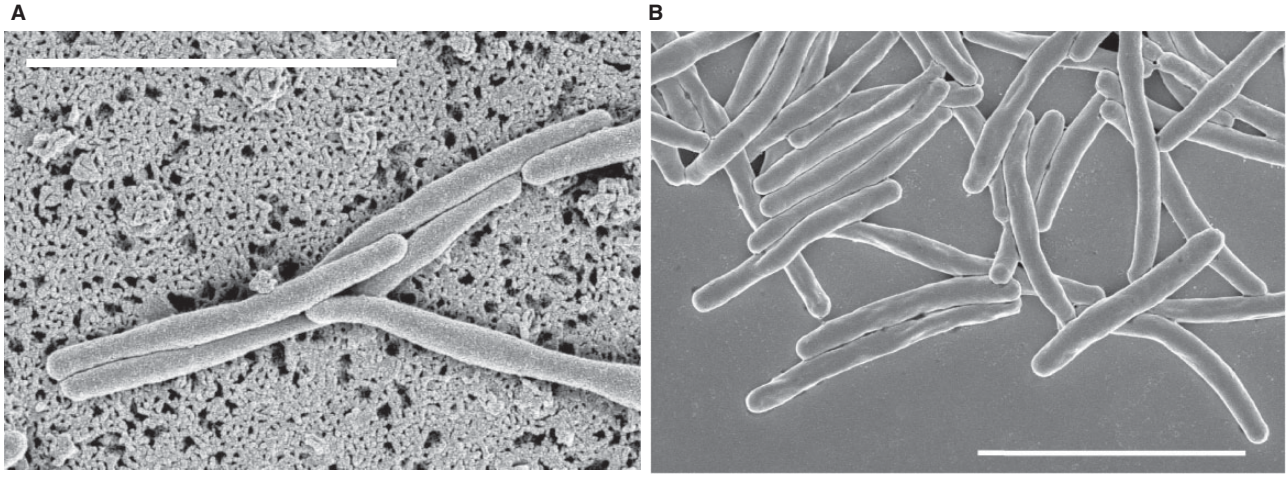

FIG. 2.—Scanning electron micrographs of typical myxobacterial cells. (*A*) strain AM401, (*B*) strain CA060A. Bars are 5 μm long.

750 genes for *Myxococcus*/*Pyxidicoccus* spp. (fig. 3*A* and *B*). Similar shaped accumulation curves were also obtained separately for the pan-genomes of the eight *Myxococcus* spp. strains and for the three *Pyxidicoccus* spp. strains (data not shown). For *Corallococcus* spp., the size of individual genomes is less variable than for *Myxococcus*/*Pyxidicoccus* spp., and they share three times more core genes than *Myxococcus*/*Pyxidicoccus* spp. (fig. 3*A* and *B*). Accessory genes are also shared to a greater extent in *Corallococcus* spp. than in *Myxococcus*/*Pyxidicoccus* species (fig. 4), with *Corallococcus* spp. having a lower proportion of genes found in single genomes, and a greater proportion of genes found in two genomes.

As the number of genomes considered is increased, the size of the pan-genome increases almost linearly for both genera (fig. 3*C* and *D*). For organisms with closed pan-genomes, the size of the pan-genome plateaus with increasing numbers of considered genomes. The linearity exhibited by figure 3*C* and *D* suggests that both the *Corallococcus* spp. and *Myxococcus*/*Pyxidicoccus* spp. pan-genomes are extremely open, with the majority of genes (about 70% and 90%, respectively, for the two genera), in the genome of each type strain belonging to the accessory genome (table 5). Fitting the curves in figure 3*C* and *D* to a power law distribution allows extrapolation of pan-genome size as a function of included genomes (table 5). For *Corallococcus* spp., it is predicted that when the genome of the 101st *Corallococcus* sp. is added, the pan-genome will increase by a further 934 genes. For *Myxococcus*/*Pyxidicoccus* spp., the 101st species would be predicted to add a further 3,529 genes to the pan-genome, reflecting the larger size of the accessory genome in this genus.

Table 3

Growth Characteristics of *Myxococcus* spp. Type Strains, *Pyxidicoccus fallax* DSM 14698^T, and Candidate Isolates

	CA032A	CA060A	*P. fallax* DSM 14698^T	AB053B	AM301	AM401	*M. fulvus* DSM 16525^T	*M. macrosporus* DSM 14697^T	*M. stipitatus* DSM 14675^T	*M. virescens* DSM 2260^T	*M. xanthus* DSM 16526^T
30 °C	++	+++	+++	+++	++	++	++	++	+++	+++	+++
35 °C	+++	+	+++	+++	−	+	++	++	++	+++	++
37 °C	+++	+	+++	+++	−	−	++	−	−	+++	−
40 °C	++	−	+	+++	−	−	+	−	−	+	−
pH 5.0	−	−	−	++	−	+	++	−	++	++	−
pH 6.0	+	+	+	+++	−	+	++	−	++	++	+
pH 7.0	++	+	++	+++	−	+	+++	++	+++	+++	++
pH 8.0	+++	+++	+++	+	++	+	+++	++	+++	+++	++
pH 9.0	+++	+++	+++	+	+	+	+++	−	+++	+++	++
1% NaCl	++	+	+++	++	+	+	+	+++	++	+++	+++
2% NaCl	+++	−	−	++	−	+	−	−	−	++	++
3% NaCl	++	−	−	++	−	+	−	−	−	+	−
4% NaCl	−	−	−	+	−	+	−	−	−	−	−

Note.—Rate of growth is indicated as "−" (no growth), "+" (slow), "++" (moderate), or "+++" (fast). Temperature dependence was tested at pH 7.8, whereas pH dependence was tested at 30 °C. NaCl tolerance was tested at 30 °C and pH 7.8 with different % NaCl concentrations (w/v).

Table 4

Biochemical Activities (A) of *Myxococcus* and *Pyxidicoccus* spp.-Type Strains and Candidate Isolates, and (B) Their Predatory Activity

A: API NE Results	**CA032A**	**CA060A**	***Pf***	**AB053B**	**AM301**	**AM401**	***Mf***	***Mm***	***Ms***	***Mv***	***Mx***
Nitrate reduction	−	−	+	−	−	−	+	−	+	−	+
Indole production	−	−	−	−	−	−	−	−	−	−	−
Glucose acidification	−	−	−	−	−	−	−	−	−	−	−
Arginine dihydrolase	+	+	+	+	+	−	−	+	−	−	−
Urease	+	+	+	+	+	+	+	+	−	+	+
Esculin hydrolysis	+	+	+	+	−	+	+	+	+	+	+
Gelatin hydrolysis	+	+	+	+	−	+	+	−	+	+	+
p-Nitrophenyl-β-D-galactopyranoside	+	+	+	+	−	+	+	−	+	+	+
Glucose assimilation	−	−	+	+	−	+	+	−	+	−	−
Arabinose assimilation	−	−	−	+	−	+	+	−	+	+	+
Mannose assimilation	−	−	−	+	−	+	+	−	+	+	−
Mannitol assimilation	−	−	−	+	−	+	+	−	+	+	+
N-Acetyl-glucosamine assimilation	−	−	−	+	−	+	+	−	+	+	−
Maltose assimilation	−	−	−	+	−	+	+	−	+	+	−
Gluconate assimilation	−	−	−	+	−	−	+	−	+	−	−
Caprate assimilation	−	−	−	+	−	−	−	−	−	−	−
Adipate assimilation	−	−	−	+	−	+	−	−	+	+	−
Malate assimilation	−	−	−	+	+	+	+	−	+	+	−
Citrate assimilation	−	−	−	+	−	−	+	−	+	+	−
Phenyl acetate assimilation	−	−	−	−	−	+	−	−	+	+	−
B: Predatory Activity	**CA032A**	**CA060A**	***Pf***	**AB053B**	**AM301**	**AM401**	***Mf***	***Mm***	***Ms***	***Mv***	***Mx***
Escherichia coli TOP10	−	−	−	−	−	−	+	−	+	+	+
Clavibacter nebraskensis DSM 7483	+	−	+	−	−	−	+	+	+	−	−
Ustilago maydis DSM 14603	−	−	−	−	−	−	+	−	+	+	+

Note.—(A) Results of API NE kits: possession of a biochemical activity is indicated with a "+" and an absence of the activity with a "−." (B) Predatory activity against three prey organisms is presented as a "+" if above the average for that prey, and "−" if below the average. *Mf*, *M. fulvus*; *Mm*, *M. macrosporus*; *Ms*, *M. stipitatus*; *Mv*, *M. virescens*; *Mx*, *M. xanthus*; *Pf*, *Pyxidicoccus fallax*.

Table 5
Pan-Genome Characteristics of *Corallococcus* spp. and *Myxococcus/Pyxidicoccus* Species

	Corallococcus spp.	*Myxococcus/Pyxidicoccus* spp.
Number of species (*n*)	10	11
Mean number of genes/genome (±standard deviation)	8,354 (±301)	8,775 (±1,276)
Pan-genome size (genes)	35,481	64,700
Mean % of core genes in each genome	29.1	8.6
Core genes (% of pan-genome)	2,428 (6.8)	755 (1.2)
Accessory genes (% of pan-genome)	33,053 (93.2)	63,945 (98.8)
Shell genes (% of pan-genome)	10,660 (30.0)	12,059 (18.6)
Cloud (% of pan-genome)	22,393 (63.1)	51,886 (80.2)
Extrapolated pan-genome, $n = 101$ (new)	151,435 (934)	424,988 (3,529)
Extrapolated pan-genome, $n = 501$ (new)	410,050 (509)	1,626,369 (2,721)
Extrapolated pan-genome, $n = 1001$ (new)	630,678 (392)	2,904,818 (2,432)
Extrapolated core genes at n=infinity	2,252	708
Named-product BGCs: core (% of total)	5 (8.5)	5 (7.7)
Named-product BGCs: shell (% of total)	28 (47.5)	34 (52.3)
Named-product BGCs: cloud (% of total)	26 (44.1)	26 (40.0)

NOTE.—The composition of each pan-genome is presented, with extrapolations to larger numbers of included genomes (*n*). The number of BGCs with similarity to those producing named compounds is also presented, and whether the BGCs are part of the core, shell, or cloud genomes.

Secondary Metabolite Gene Clusters Are Enriched in the Shell Pan-Genome

The antimicrobial secondary metabolites secreted by myxobacteria are important determinants of their predatory activity, and there is also considerable pharmaceutical interest in myxobacterial metabolites, which can exhibit broader (e.g., cytotoxic) activities. BGCs were identified in the genome of each *Corallococcus* spp. and *Myxococcus/Pyxidicoccus* spp. type strain using antiSMASH 5.1.2 (Blin et al. 2019). Many of the BGCs identified in this way exhibited similarity to BGCs known to produce particular (named) metabolites (supplementary file 2, Supplementary Material online).

On average, each *Corallococcus* spp. genome included 60 BGCs (including those predicted to produce 19 named products), whereas each *Myxococcus/Pyxidicoccus* spp. genome had 46 BGCs (predicted to produce 18 named products). The *Corallococcus* spp. BGCs were predicted to produce metabolites belonging to 31 compound classes, whereas *Myxococcus/Pyxidicoccus* spp. BGCs encoded 34 compound classes. As can be seen in supplementary file 2, Supplementary Material online, for both genera, nonribosomal peptide synthases (nrps) were particularly common (29 per genome and 19 per genome for *Corallococcus* and *Myxococcus/Pyxidicoccus*, respectively), whereas hybrid products belonging to multiple compound classes (e.g., nrps-arylpolyene) were also prevalent (12 per genome for both *Corallococcus* spp. and *Myxococcus/Pyxidicoccus* spp.).

The distribution of "named BGCs" (those producing "named metabolites") was investigated, and for each genus, BGCs were defined as core (present in every genome), shell (present in more than one, but not all genomes), or cloud (present in only one genome). For both genera, there were (the same) five core BGCs, predicted to produce alkylpyrone-407/393, carotenoid, geosmin, myxoprincomide-c506, and VEPE/AEPE/TG-1. For *Corallococcus* spp., there were 28 shell and 26 cloud named BGCs, whereas *Myxococcus/Pyxidicoccus* spp. had 34 shell and 26 cloud named BGCs (table 5).

For both genera, the proportion of named BGCs found in the shell was considerably enriched compared with that of the whole pan-genome (47–52% compared with 19–30%), whereas the cloud pan-genome was relatively impoverished in named BGCs (40–44% compared with 63–80%). In other words, the BGCs of the accessory pan-genome are found in multiple genomes more frequently than would be expected by chance, implying there is a selective pressure for retention of those BGCs, presumably because of advantages they might convey during predation.

Discussion

Taxonomic Classification of Myxobacterial Species on the Basis of Genomic and Phenotypic Diversity

Classification is the process of categorizing variation between organisms to produce discrete taxonomic groupings based on shared characteristics. Myxobacterial taxonomists have traditionally employed a polyphasic approach for classification using multiple measures, as no simple set of measurements are able to consistently delineate species across the Myxococcales (Mohr et al. 2018). This is in part due to the complexity of the myxobacterial life-cycle, which results in phenotypic measurements being exquisitely sensitive to variations in media and other experimental conditions (Garcia et al. 2010). However, there are also no clear criteria regarding which phenotypes should differ, and by what extent, in order to define novel species.

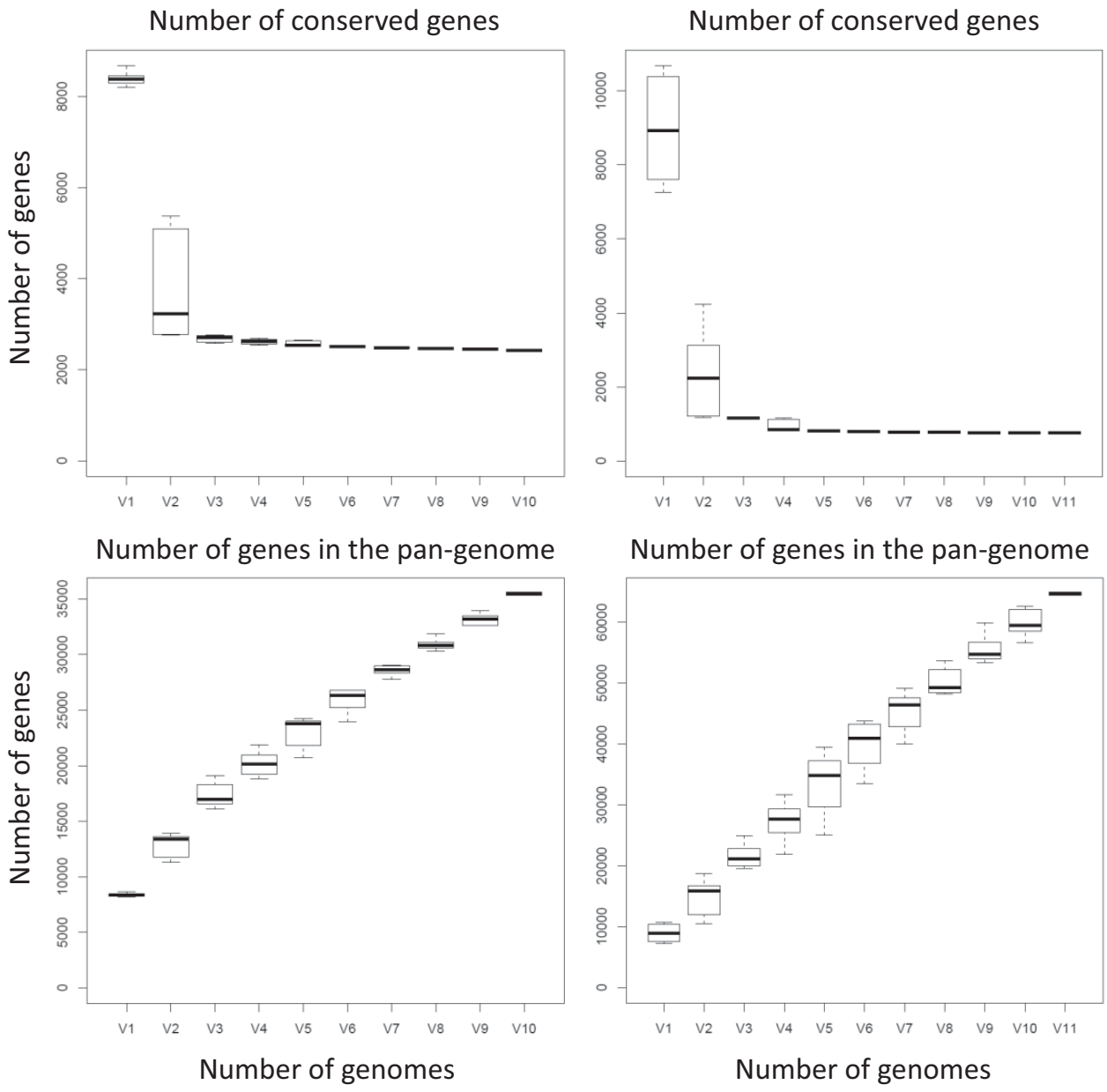

Fig. 3.—The *Corallococcus* (left) and *Myxococcus/Pyxidicoccus* (right) core genome (top) and pan-genome (bottom) as a function of the number of genomes included (V1–V11). The boxes show the median number of genes ± 1 SD, whiskers show ±2 SD.

An alternative approach is to use gene and genome sequences to underpin the proposal of novel species. The greater the number of genes used for phylogenetic reconstruction, the more highly resolved the resulting trees can be, and using the entire core genome is therefore maximally informative (Richter and Rosselló-Móra 2009; Chun et al. 2018). The data obtained by sequencing are also unambiguous, and unaffected by experimental conditions. A genome-based approach also has the advantage of being driven by shared evolutionary heritage rather than shared phenotypic properties which can emerge from convergent evolution.

Classification of organisms into coherent taxa is based on shared genotypes and/or phenotypes, but within each level of classification there remains diversity among the organisms of each taxon, which is greater at higher levels of classification. In bacteria, genotypic diversity can be measured in various ways. The sequence similarity of conserved genes provides one measure of diversity, which is captured by metrics such as the ANI and 16S rRNA gene phylogenetics to assign organisms to the same or different species. However, the commonality of genes is another form of diversity exhibited by organisms with open pan-genomes (where the pan-genome

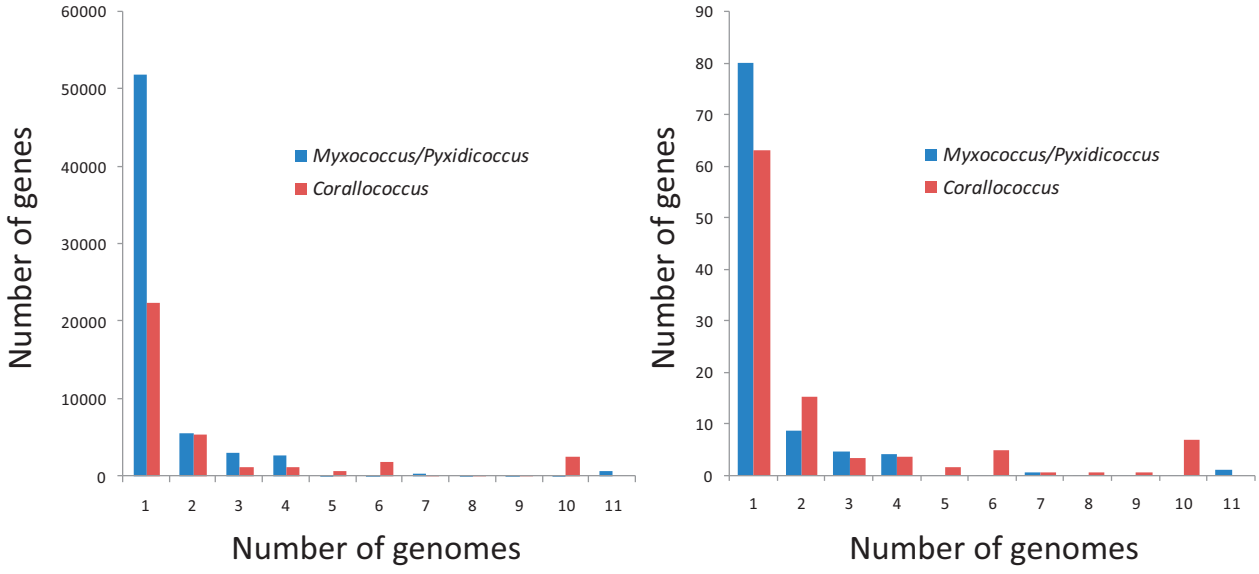

Fig. 4.—*Corallococcus* spp. and *Myxococcus/Pyxidicoccus* spp. genes as a function of the number of genomes each gene is found in. Presented as both the number of genes (left) and the percentage of the pan-genome (right).

increases in size as more genomes are included) (fig. 3, bottom panels). The presence/absence of accessory genes represents a fundamentally different type of diversity from the sequence similarity of core genes. For instance, *Myxococcus/Pyxidicoccus* spp. type strains only share around 20% of their genes (fig. 4); however, the genes, which are shared, share an average ANI of 84%.

In this study, we unambiguously identified five novel species within the Myxococcaceae. Results from a polyphasic phenotypic approach were consistent with taxonomic assignment based on genome sequences, even though subsequent pan-genome analysis revealed that only a small portion of each genome was shared between the species within each genus. Previous studies had shown that myxobacterial pan-genomes are large and open (Livingstone, Morphew, Whitworth 2018; Zwarycz et al. 2020); however, the paucity of genome sequences available made comparing the pan-genomes of different species and genera difficult. For instance, cloud genes cannot be identified unless at least ten genomes are considered. In addition, it is known that the phylogenetic distance of analyzed genomes affects apparent pan-genome properties (Park et al. 2019), so it is important to control for relatedness of all genomes included (hence, our choice to use single genomes from different species in this study).

The Pan-Genomes of Myxobacterial Genera Exhibit Differences in Gene Diversity

Corallococcus spp. genomes are less diverse than those of *Myxococcus/Pyxidicoccus* spp.—their core genes are more similar as evidenced by higher ANI scores (intragenus comparisons give ANI values of 88% [±3%] and 84% [±3%], respectively), and their accessory genes are shared to a greater extent (figs. 3 and 4). The core genome of *Corallococcus* spp. is three times larger than that of *Myxococcus/Pyxidicoccus* spp., with a correspondingly smaller accessory pan-genome (table 5). These properties jointly contribute to the pan-genome of *Myxococcus/Pyxidicoccus* spp. being more open than that of *Corallococcus* spp. (growing faster as more genomes are added).

It is difficult to even speculate why *Myxococcus/Pyxidicoccus* spp. genomes might be more diverse than those of *Corallococcus* species. Previous work has suggested that myxobacterial genomes might be subject to relatively weak purifying selection, allowing accumulation of large accessory genomes filled with horizontally acquired genes, potentially giving rise to the broad prey range exhibited by myxobacteria (Sutton et al. 2019). However, it is not clear why some predatory genera might benefit from having a larger accessory pan-genome than other predatory genera—perhaps it increases the chances that strains will have "rare gems" with potent activity among their accessory genes (Vos et al. 2015). When more species are identified, it would be interesting to see whether non-myxobacterial wolf-pack predators like *Herpetosiphon* spp. might also have large accessory pan-genomes and how they compare with those of myxobacteria.

The *Corallococcus* spp. and *Myxococcus/Pyxidicoccus* spp. Pan-Genomes Are Unusually Open

Most pan-genome studies have used genomes from individual species of bacteria. However, an analysis of 23 strains from the *Shewanella* genus showed that the core represented

around 40% of each genome (Zhong et al. 2018), relatively high compared with the 29% and 9% we observe for *Corallococcus* spp. and *Myxococcus/Pyxidicoccus* spp., respectively. Studies within single non-myxobacterial species have shown that the core genome represents between 50% and 90% of the genome (Tettelin et al. 2005; Park et al. 2019), averaging roughly 70%. A pan-genome analysis of ten *M. xanthus* strains found that on average core genes accounted for 74% of each genome (Zwarycz et al. 2020), which therefore seems typical for a within-species bacterial pan-genome.

The proportion of the genome composed of core genes is thus substantially higher for *M. xanthus* than for the *Myxococcus/Pyxidicoccus* genus (74% vs. 9%, respectively), implying that each species has a large complement of species-specific core genes (about 65% of each genome), which are generally not shared by other species within the genus. However, this may be a peculiarity of *M. xanthus* and other species should be isolated and sequenced in depth to allow pan-genome analysis of further myxobacterial species.

Further Taxonomic Considerations

We used ANI calculations, dDDH calculations, and phylogenetic comparisons to confirm the novelty of five strains belonging to the Myxococcaceae. With a cut-off of 95% ANI and 70% dDDH representing membership of the same species, only two organisms gave ANI values above 95% and dDDH values above 70%; *M. xanthus* DSM 16526^T and *M. virescens* DSM 2260^T (table 2), indicating they belong to a single species. Such a conclusion was supported by phylogenetic analysis of 16S rRNA gene sequences (fig. 1*A*), with complete 16S gene sequences showing 99.9% identity (one nucleotide difference out of 1,493). As *M. virescens* (Thaxter 1892) has priority over *M. xanthus* (Beebe 1941), if both species are actually a single species, *M. xanthus* should be considered a synonym of *M. virescens* rather than a correct name. However, it is also possible that *M. virescens* DSM 2260^T has been misidentified and should actually be *M. xanthus* DSM 2260. Other isolates classified as *M. virescens* should be genome sequenced and characterized to see whether *M. virescens* is a discrete species from *M. xanthus*. It should also be noted that we nevertheless observed phenotypic differences between *M. xanthus* DSM 16526^T and *M. virescens* DSM 2260^T (tables 3 and 4), highlighting some of the benefits of an objective genomic based system for taxonomic assignment.

Our results also show that *Pyxidicoccus* is not a separate genus from *Myxococcus*. Previous phylogenetic analysis of 16S rRNA gene sequences had shown *Pyxidicoccus* branching within the *Myxococcus* clade (Garcia et al. 2010), agreeing with our own analysis (fig. 1*A*). Indeed, whether considering 16S rRNA gene sequences, ANI, or dDDH values, *P. fallax* is as distinct from *Myxococcus* spp. as *Myxococcus* spp. are from each other (table 2 and fig. 1*B* and *C*). In contrast, *Corallococcus* spp. are more different from *Myxococcus/Pyxidicoccus* spp. (ANI values below 81% and dDDH values below 22%) than any pair of *Myxococcus/Pyxidicoccus* spp. are from each other. The *Pyxidicoccus* genus was proposed in 2007 (Reichenbach 2007), after the publication of the *Myxococcus* genus (Thaxter 1892); therefore, *Pyxidicoccus* should be considered a synonym of *Myxococcus*. Sangal et al. (2016) have suggested that an ANI value of 75% could be appropriate for distinguishing between bacterial genera, which implies that it might even be appropriate to consider *Corallococcus*, *Myxococcus*, and *Pyxidicoccus* as a single genus (*Myxococcus*), and we would encourage further investigations into the taxonomic relationships between these genera.

It should be noted that the increased genomic diversity observed for *Myxococcus/Pyxidicoccus* spp. compared with *Corallococcus* spp. is not due to the inappropriate inclusion of strains from more than one genus (if considering *Pyxidicoccus* as a separate genus). Pairwise comparisons within *Myxococcus/Pyxidiccoccus* spp. give a mean ANI value of 84% (standard deviation 3%), and this value does not change if *P. fallax* (or *P. fallax*, CA032A, and CA060A) are omitted from consideration.

Implications for Natural Products Discovery

Only a small proportion of BGCs (8–9%) were found to be core components of the genomes analyzed here. However, BGCs were found to be relatively enriched in the shell (rather than cloud) pan-genome compared with non-BGC genes, implying the presence of selective pressures for the evolutionary retention of BGCs. Nevertheless, the vast majority of BGCs were found in the accessory pan-genome, which suggests that they either have redundant or contingent functions or provide little to no selective advantage. Perhaps, to more effectively identify functionally important BGCs and the organisms possessing them, we should consider enrichment of particular BGCs within the accessory pan-genome rather than taking an adaptationist functional genomics approach to BGC discovery.

More than two-thirds of the BGCs found in each genus were not found to be similar to previously characterized BGCs producing named metabolites, suggesting that despite decades of screening natural isolates for bioactives, there is considerable BGC diversity remaining unexplored in the Myxococcaceae. As the *Myxococcus/Pyxidicoccus* genus had a greater complement and diversity of BGCs than *Corallococcus* spp., it might make sense to focus future isolation efforts on members of that genus.

It seems likely that continued sampling will continue to bring to light further novel species of Myxococcaceae, although as more species are described, the likelihood of finding a member of an undescribed species will become

progressively lower. This likelihood will also be affected by the relative abundance of particular species. Within our culture collection of nearly 200 myxobacterial isolates, some species dominate (for instance around 50% of our *Corallococcus* spp. isolates are *C. Corallococcus exiguus* and about 50% of our *Myxococcus* spp. isolates are *M. xanthus*), probably reflecting their relative abundance in nature, and making isolation experiments increasingly likely to reisolate examples of abundant known species compared with novel minority species. Consistent with this observation, the five novel species described here include no other examples from among the 90 sequenced isolates of the Livingstone et al. (2017) collection. It would be interesting to see whether any currently unassigned 16S rRNA gene sequences in the NCBI database belong to these five new species.

With at least ten species now identified in each of the *Corallococcus* and *Myxococcus*/*Pyxidicoccus* genera, it would be interesting to investigate other aspects of evolution and ecology in each genus. For instance, are the same sets of genes subject to positive selection in the two genera, do they occupy different ecological niches, and how does their geographic distribution relate to environmental parameters and/or microbiome composition? Understanding the myxobacterial pan-genome and its constituent genus and species-specific marker genes should also allow a more fine-grained identification of Myxococcaceal strains within metagenomic data sets. Potentially, we could then direct bioprospecting efforts toward the environments/locations likely to contain the most diverse organisms, with the greatest chance of producing novel bioactive compounds.

Species Descriptions

Myxococcus eversor sp. nov.

Myxococcus eversor (e.ver'sor L. masc. n. *eversor* the destroyer, reflecting its destruction of prey cells).

Vegetative cells are Gram-negative bacilli tapering slightly at the ends, measuring 0.6–0.7 μm × 3.0–8.0 μm in electron micrographs. Colonies exhibit swarming motility and appear pale brown on VY-2 agar (w/v 0.5% dried baker's yeast, 0.1% $CaCl_2 \cdot 2H_2O$, and 1.5% agar). Fruiting bodies are irregular spheroids, orange in color. Aerobic growth was observed at 30–40 °C, at pH 5.0–9.0, and with the addition of 1–4% NaCl. Hydrolyzes arginine, esculin, gelatin, *p*-nitrophenyl-β-D-galactopyranoside, and urea. Assimilates *N*-acetyl-glucosamine, adipate, arabinose, caprate, citrate, gluconate, glucose, malate, maltose, mannitol, mannose, and phenyl acetate. Cells prey with low efficiency upon *E. coli* TOP10, *Cl. nebraskensis* DSM 7483, and *U. maydis* DSM 14603.

DNA GC content is 68.9 mol%. The draft genome sequence of AB053B^T is available from GenBank (accession JAAIXY01). The type strain (AB053B^T = NCCB 100767^T = NBRC 114350^T) was isolated from soil collected from Aberystwyth University, UK (gridref 52.41°N 4.08°W).

Myxococcus llanfairpwllgwyngyllgo gerychwyrndrobwllllantysiliogogogochensis sp. nov.

Myxococcus llanfairpwllgwyngyllgogerychwyrndrobwllllantysiliogogogochensis, (llan.fair.pwll.gwyn.gyll.gog.er.ych.wyrn.dro.bwllll.ant.ysil.iog.ogogoch.en'sis. N.L. masc. adj. llanfairpwllgwyngyllgogerychwyrndrobwllllantysiliogogogochensis, pertaining to llanfairpwllgwyngyllgogerychwyrndrobwllllantysiliogogogoch, reflecting its isolation from soil collected in that parish [gridref 53.22°N 4.19°W]).

Vegetative cells are Gram-negative bacilli tapering slightly at the ends, measuring 0.4–0.6 μm × 4.0–7.0 μm in electron micrographs. Colonies exhibit swarming motility and appear pale brown on VY-2 agar (w/v 0.5% dried baker's yeast, 0.1% $CaCl_2 \cdot 2H_2O$, and 1.5% agar). Fruiting bodies are irregular spheroids, orange in color. Aerobic growth was observed at 30 and 35 °C and at pH 5.0–9.0. Growth was unaffected by the addition of 1–4% NaCl. Hydrolyzes esculin, gelatin, *p*-nitrophenyl-β-D-galactopyranoside, and urea. Assimilates *N*-acetyl-glucosamine, adipate, arabinose, glucose, malate, maltose, mannitol, mannose, and phenyl acetate. Cells prey with low efficiency upon *E. coli* TOP10, *Cl. nebraskensis* DSM 7483, and *U. maydis*. DNA GC content is 68.7 mol%. The draft genome sequence of AM401^T is available from GenBank (accession VIFM01). The type strain (AM401^T = NBRC 114351^T = NCCB 100770^T) was isolated from soil collected in the parish of Llanfairpwllgwyngyllgo gerychwyrndrobwllllantysiliogogogoch, UK (gridref 53.22°N 4.19°W).

Myxococcus vastator sp. nov.

Myxococcus vastator (vas.ta'tor L. masc. n. *vastator* the ravager, after its ability to devastate colonies of prey cells).

Vegetative cells are Gram-negative bacilli tapering slightly at the ends, measuring 0.6–0.7 μm × 3.0–6.0 μm in electron micrographs. Colonies exhibit swarming motility and appear pale brown on VY-2 agar (w/v 0.5% dried baker's yeast, 0.1% $CaCl_2 \cdot 2H_2O$, and 1.5% agar). Fruiting bodies are irregular spheroids, orange in color. Aerobic growth was observed at 30 °C and at pH 8.0–9.0. Growth was unaffected by the addition of 1% NaCl. Hydrolyzes arginine and urea. Assimilates malate. Cells prey with low efficiency upon *E. coli* TOP10, *Cl. nebraskensis* DSM 7483, and *U. maydis* DSM 14603.

DNA GC content is 69.9 mol%. The draft genome sequence of AM301^T is available from GenBank (accession JAAIYB01). The type strain (AM301^T = NCCB 100768^T = NBRC 114352^T) was isolated from soil collected in the parish

of Llanfairpwllgwyngyllgogerychwyrndrobwlll lantysiliogogogoch, UK (gridref 53.22°N 4.19°W).

Pyxidicoccus caerfyrddinensis sp. nov.

Pyxidicoccus caerfyrddinensis (caer.fyrdd.in.en'sis N.L. masc. adj. *caerfyrddinensis* from Caerfyrddin, reflecting its isolation from soil sampled near Carmarthen [the Anglicized name for Caerfyrddin], Wales [51.86°N 4.31°W]).

Vegetative cells are Gram-negative bacilli tapering slightly at the ends, measuring 0.7–0.8 μm × 3.0–8.0 μm in electron micrographs. Colonies exhibit swarming motility and appear pale brown on VY-2 agar (w/v 0.5% dried baker's yeast, 0.1% $CaCl_2{\cdot}2H_2O$, and 1.5% agar). Fruiting bodies are irregular spheroids, orange in color. Aerobic growth was observed at 30–40 °C and at pH 6.0–9.0. Growth was unaffected by the addition of 1–3% NaCl. Hydrolyzes arginine, esculin, gelatin, *p*-nitrophenyl-*β*-D-galactopyranoside, and urea. Cells prey efficiently on *Cl. nebraskensis* DSM 7483, and with low efficiency upon *E. coli* TOP10 and *U. maydis* DSM 14603.

DNA GC content is 70.2 mol%. The draft genome sequence of CA032A^T is available from GenBank (accession JAAIYA01). The type strain (CA032A^T = NCCB 100776^T = NBRC 114353^T) was isolated from soil collected in Carmarthen, UK (gridref 51.86°N 4.31°W).

Pyxidicoccus trucidator sp. nov.

Pyxidicoccus trucidator (tru.ci'da.tor L. masc. n. *trucidator* the slaughterer, after its widespread cytotoxicity).

Vegetative cells are Gram-negative bacilli tapering slightly at the ends, measuring 0.5–0.6 μm × 3.0–6.0 μm in electron micrographs. Colonies exhibit swarming motility and appear pale brown on VY-2 agar (w/v 0.5% dried baker's yeast, 0.1% $CaCl_2{\cdot}2H_2O$, and 1.5% agar). Fruiting bodies are irregular spheroids, orange in color. Aerobic growth was observed at 30–37 °C, at pH 6.0–9.0, and in 0–1% NaCl. Hydrolyzes arginine, esculin, gelatin, *p*-nitrophenyl-*β*-D-galactopyranoside, and urea. Cells prey with low efficiency upon *E. coli* TOP10, *Cl. nebraskensis* DSM 7483, and *U. maydis* DSM 14603.

DNA GC content is 70.3 mol%. The draft genome sequence of CA060A^T is available from GenBank (accession JAAIXZ01). The type strain (CA060A^T = NCCB 100777^T = NBRC 114505^T) was isolated from soil collected in Llansteffan, UK (gridref 51.77°N 4.39°W).

Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online.

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Author Contributions

P.G.L. isolated the strains. J.C., N.Sp., and N.Sy. cultured and characterized the strains experimentally, with guidance from D.E.W. and P.G.L. J.C. and N.Sp. also characterized the previously identified *Myxococcus* spp. type strains. N.Sy. performed pan-genome analyses. A.R.C. operated the electron microscope. D.E.W. drafted the manuscript, which all authors edited.

Data Availability

For each of the five novel species reported here, the strains and their genome sequence data are available through culture collections and GenBank, as stated in each species description above.

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