Characterisation of the UK honey bee (*Apis mellifera*) metagenome.

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Abstract

The European honey bee (*Apis mellifera*) plays a major role in pollination and food production, but is under threat from emerging pathogens and agro-environmental insults. As with other organisms, honey bee health is a complex product of environment, host genetics and associated microbes (commensal, opportunistic and pathogenic). Improved understanding of bee genetics and their molecular ecology can help manage modern challenges to bee health and production. Sampling bee and cobiont genomes, we characterised the metagenome of 19 honey bee colonies across Britain. Low heterozygosity was observed in bees from many Scottish colonies, sharing high similarity to the native dark bee, *A. mellifera mellifera*. Apiaries exhibited high diversity in the composition and relative abundance of individual microbiome taxa. Most non-bee sequences derived from known honey bee commensal bacteria or known pathogens, e.g. *Lotmaria passim* (Trypanosomatidae), and *Nosema spp.* (Microsporidia). However, DNA was also detected from numerous additional bacterial, plant (food source), protozoan and metazoan organisms. To classify sequences from cobionts lacking genomic information, we developed a novel network analysis approach clustering orphan contigs, allowing the identification of a pathogenic gregarine. Our analyses demonstrate the power of high-throughput, directed metagenomics in agroecosystems identifying potential threats to honey bees present in their microbiota.

Introduction

The European honey bee, *Apis mellifera* Linnaeus, has a global distribution and a major role in pollination and food production (1). Like other pollinators, honey bee populations face multiple threats. The UN's food and agriculture organisation estimate that 75% of all pollinators are in decline globally and their numbers have dropped by about one third in the past decade (2). Whilst flowering crops benefit greatly from a diversity of insect pollinators (3), managed honey bees are a major global contributor, providing nearly half of the service to all insect-pollinated crops on Earth (4, 5). Despite the recent increase in non-commercial beekeeping, the number of managed honey bee colonies is growing more slowly than agricultural demand for pollination (6). The decline in pollinators is not thought to be caused by a single factor but may be driven by a combination of habitat fragmentation, agricultural intensification, pesticide residue accumulation, new honey bee pests and diseases, and suboptimal beekeeping practices (7-9). Trade in honey bees from different regions of the globe have unquestionably contributed to a rise in infectious disease and there may be transmission between honey bees and wild pollinators (10, 11).

In the UK the genetic structure of honey bee populations has undergone large changes over the last 100 years. The native M-lineage subspecies, *A. m. mellifera,* had predominated in the UK, but the population was decimated in the early 20th century by a combination of poor weather and chronic bee paralysis virus, thought to have been caused by Isle of Wight disease (12). Following this, the practice of bee importation increased dramatically. In the UK today there is a growing industry that imports bees from mainland Europe, particularly the Italian honey bee (*A. m. ligustica*) and Carniolan honey bee (*A. m. carnica*), both C-lineage subspecies. Importation of queens has for a long time been used as a means to compensate for the loss of colonies and the Southern European strains are often viewed as a means to improve honey production. It had been assumed that the native UK bee was extinct, but new molecular studies have shown that colonies robustly assigned to *A. m. mellifera* still exist in Northern Europe (13). In the UK the genetic diversity of honey bee populations is poorly understood. The genetic makeup of bee populations not only influences production traits and the ability to survive under less favourable conditions, but also plays a vital role in disease resistance (14). However, the

74 movement of honey bees across the globe has unquestionably contributed to the
75 spread of infectious diseases, which may also be transmitted to wild pollinators (10,
76 11).

77 In the UK, the health of honey bees is under threat from a range of native and non-
78 native bacterial, fungal and viral pathogens. While known 'notifiable diseases' can be
79 risk assessed and regulated by law, emergent diseases such as *Nosema ceranae*
80 (15) may be spread globally before they have been properly identified and risk
81 assessed. Nosemosis is one of the most prevalent honey bee diseases and is
82 caused by two species of microsporidia, *Nosema apis* and *Nosema ceranae*, that
83 parasitize the ventriculum (midgut). Although infected bees often show no clear
84 symptoms, heavy infections can result in a broad range of detrimental effects (16-
85 21). *N. ceranae,* a native parasite of the Asiatic honey bee (*Apis cerana*), has been
86 detected in *Apis mellifera* samples from Uruguay predating 1990 but is now present
87 in *Apis mellifera* worldwide (15). Notifiable diseases, American foulbrood (AFB) and
88 European foulbrood (EFB), are caused by the non-native bacteria *Paenibacillus*
89 *larvae* and native *Melissococcus plutonius*, respectively (22, 23). Acarine disease is
90 caused by a mite found throughout Britain which infests the trachea of honey bees
91 (24). Protozoans such as gregarines and the emergent trypanosomatid *Lotmaria*
92 *passim*, also infect honey bees. The most devastating of all pathogenic species in
93 recent years is the hemophagous mite *Varroa destructor,* which shifted hosts from *A.*
94 *cerana* to *A. mellifera* sometime in the first half of the 20th century (25). *Varroa* mites
95 feed on the haemolymph of both larval and adult stages of the honey bee. More
96 importantly, *V. destructor* transmits several bee viruses, generating epidemics that
97 kill colonies within two to three years unless the *Varroa* population is kept under
98 control. Among the most important and lethal viruses in this regard are deformed
99 wing virus (DWV) (26), acute bee paralysis virus complex (ABPV), Kashmir bee virus
100 (KBV), and Israeli acute paralysis virus (IAPV) (27). Sacbrood virus (SBV) can also
101 be transmitted but without major epidemic consequences and is primarily indirectly
102 affected by *Varroa* (25, 28, 29).

103 The core commensal microbiome can mediate disease susceptibility and the internal
104 ecology of the host can greatly affect disease outcome (30). In addition to
105 immunological health and essential nutrient provision, microbial metabolism affects

the growth, behaviour and hormonal signalling of honey bees (31). Unlike most host species, the core microbiota of the honey bee has relatively little diversity (32-38). *Snodgrasella alvi* (Betaproteobacteria), *Gilliamella apicola* (Gammaproteobacteria), two *Lactobacillus* taxa (Firm-4 and Firm-5) (34, 35), and *Bifidobacterium asteroides* are common and abundant (39, 40). There are at least four less common species: *Frischella perrara* (41), *Bartonella apis* (42), *Parasaccharibacter apium* (37) and Gluconobacter-related species group Alpha2.1 (35). Metagenomic analyses have revealed high between-isolate genetic diversity in honey bee microbiotal taxa, suggesting they comprise clusters of related taxa (43). These bacteria maintain gut physiochemical conditions and aid their host in the digestion and metabolism of nutrients, neutralisation of toxins, and resistance to parasites (44-46). *Gilliamella* species digest pectin from pollen, and the *Lactobacillus* species inhibit the growth of foulbrood bacteria (47). However, *F. perrara* may cause a widespread scab phenotype in the gut (48). A negative correlation was found between the presence of *Snodgrasella alvi* and pathogenic *Crithidia* in bees (49), but pre-treatment of honey bees with *S. alvi* prior to challenge with *Lotmaria passim* (an *A. mellifera* pathogen closely related to *Crithidia*) resulted in greater levels of *L. passim* compared to bees which were not pre-treated (50). Thus, commensal microbiome species can have beneficial, mutual or parasitic relationships with their hosts, and in particular, different combinations of species – different microbiota communities – may be associated with variations in honey bee health.

With recent significant reductions in the cost of high throughput sequencing, metagenomics could be a useful tool for analysing genetic lineage, gut health and pathogen load as part of routine testing and/or monitoring imports for novel pathogens. Here, to establish baseline figures and test the suitability of this approach, we applied a novel network analysis framework together with deep sequencing of the honey bee metagenome, examining the genomes of honey bees and their symbiotic and pathogenic cobionts in UK apiaries.

Results

Metagenome sequencing of honey bees and their cobionts

We performed full metagenomic sequencing of 19 samples of UK honey bees (**Supplementary Table 1**). Samples were obtained from hives located across Scotland and England (**Fig. 1a**), each sample comprising of 16 workers collected from a single colony. Duplicates of samples 1-4 were analysed at a lower sequencing coverage to assess cobiont and genomic variant discovery. While the sample size was limited, the colonies sequenced were selected as representative of the phenotypic diversity of honey bees currently managed by UK apiarists. Notably, representatives of the Buckfast bee and the Colonsay "native" black bee lines were included in the sampling. The entire thorax and abdomen was processed for genome sequencing, thus including gut microorganisms, organisms attached to the outside of the bees, and haemolymph/tissue parasites. Between 4.5 and 12.5 million 125 base paired-end reads were generated per sample on the Illumina HiSeq 2500, equivalent to between 17- and 50-fold coverage of the honey bee genome (Amel 4.5).

Genomic diversity of sampled honey bees

DNA sequence data were mapped onto the honey bee reference genome (version Amel 4.5 (51)) and variants identified. Overall 3,940,467 sites were called as polymorphic, ranging from 962,775 to 2,586,224 single nucleotide variants (SNVs) per sample (**Fig. 1b**). A correlation graph derived from a matrix of identity-by-state (IBS) at each variant position for all samples was used to define related groups of samples (**Fig. 1c**). Group 1, which includes the native black bee sample from Colonsay (samples 2 and 9), was less heterozygous than Group 2 (**Fig. 1d**). ADMIXTURE (62) analyses were used to explore population subdivision in the data following removal of SNVs in linkage disequilibrium. ADMIXTURE cross-validation (CV) error values increased as the number of populations (K) assumed to be contributing to the variation were increased (K=1, CV= 0.562; K=2, CV= 0.601; K=3, CV= 0.712; K=4, CV= 0.853; K=5, CV= 1.007). At K=2 the Buckfast (samples 3 and 10) and Carniolan (samples 4 and 11) C lineage samples were distinguished from the M lineage *A. m. mellifera* samples, while K=3 further discerns the "native" *A. m.*

mellifera sampled from Colonsay (samples 2 and 9), the Buckfast sample at K = 4 and the *A. m. mellifera* breeding project (samples 1 and 8) at K = 5 (**Fig. 1e**).

ADMIXTURE was originally designed to estimate ancestry in unrelated individuals rather than pooled DNA from several individuals, as analysed here. To address this, genotypes were simulated for 10 individuals per pooled DNA sample, using allele sequence depth to estimate allele frequency under an assumption of Hardy-Weinberg equilibrium and analysed using ADMIXTURE. The CV error values decreased as K was increased (K=1, CV= 0.980; K=2, CV= 0.835; K=3, CV= 0.795; K=4, CV= 0.763; K=5, CV= 0.736). At K≤3 the simulated data results were consistent with those from the actual pooled genotypes, while K=4 distinguished samples from the *A. m. mellifera* breeding project (samples 1 and 8), and K=5 assigned a distinct genetic background to bees sampled from Wigtownshire (sample 15) (**Fig. 1e**). k-nearest neighbour (kNN) network analysis of the pooled genotype data (63) also identified 2 clusters, separating C and M lineage samples in the same manner as the ADMIXTURE analyses. Together, these results support a model of two genetic backgrounds in the UK bee populations sampled, most likely representing the C and M lineages, with evidence of a distinct *A. m. mellifera* background in bees originating from Colonsay and other areas of Scotland, and differentiation of Buckfast and Carniolan bees (**Fig. 1f**).

The microbiome of honey bees

The majority of the data (~90% of reads) from each sample mapped to the honey bee reference genome. Reads that did not map to the honey bee reference were collated and used for a metagenomic assembly. This resulted in over 35,000 contigs greater than 1 kb in length. Contigs were assigned to a taxonomic group by comparison to a series of curated databases in a defined order (**Fig. 2a**) using BlobTools (52). First, contigs were compared to the bee cobiont sequence data in the HoloBee Database (v2016.1) (53), followed by genomes and proteomes of species identified as being bee-associated (54, 55), and finally by comparison of contigs against the NCBI Nucleotide and UniProt Reference Proteome databases. Patterns of coverage, GC% and taxonomic annotation of contigs were explored to identify likely genomic compartments present (**Fig. 2b,c**). We discarded contigs with

read coverage lower than 1, as these were likely an artefact of pooling reads, yielding a final set 31,386 metagenome contigs, spanning 140 Mb. Taxon assignments are summarised in **Supplementary Table 2**. Correlation graphs were constructed based on the coverage or contribution to each contig from each sample. Clustering samples in this manner did not recapitulate their clustering by honey bee genome SNVs (**Fig. 2d)**. A correlation graph was also constructed where nodes represented individual contigs (**Fig. 3**). A high correlation threshold (r = 0.99) was used, which meant that 35% of the contigs were unconnected. The highly structured multi-component graph was subdivided using the MCL algorithm (56) into clusters of contigs whose abundance across the samples was very similar. Many of these clusters were made up of contigs derived from the same species or in a number of cases from strongly co-occurring species.

Rarefaction analysis of ribosomal RNA sequences present in the assembled data was used to estimate the species richness discovered as a function of sequencing depth (**Supplementary Fig. 1**). While there was variation between samples in terms of species richness at all sequencing depths, even the lowest coverage achieved (17x reference genome coverage) was likely to be sufficient to capture most *A. mellifera* cobionts present.

We examined clusters on the graph further. One (**Fig. 4a**) contained 1.33 Mb of sequence, most of which had no match in public databases, but contained some contigs that had significant similarity to sequences from other *Apis* species (**Fig. 4b**). The number of reads mapping to these contigs was proportional to the depth of honey bee genome sequencing (**Fig. 4c**) and we infer that they likely represent reads from true *A. mellifera* genome fragments not present in the honey bee reference genome (**Fig. 4d**). Others in this cluster, spanning 0.01 Mb, matched sequences from *Ascophaera apis* (chalkbrood), an endemic fungal associate of honey bees (57).

Most of the other groups of contigs could be assigned to cobiont organisms. The contribution of non-*A. mellifera* reads varied between samples, a pattern that may be partly explained by the presence in some samples of eukaryotic pathogens such as *Nosema* microsporidians and the trypanosomatid *L. passim*, which have larger

genomes. The most abundant non-pathogenic bacterial cobionts identified were *Gilliamella apicola, Bartonella apis, Frischella perrara, Snodgrassella alvi,* "Firm-4" firmicutes (58) (*Lactobacillus mellis* and *Lactobacillus mellifer),* "Firm-5" firmicutes (58) (*Lactobacillus melliventris*, *Lactobacillus kimbladii, Lactobacillus kullabergensis, Lactobacillus sp. wkB8, Lactobacillus helsingborgensis* and *Lactobacillus sp. wkB10), Lactobacillus kunkeei* and *Bifidobacterium asteroides* (**Supplementary Table 2**). Each species varied in its abundance across the samples. In some nominal species, contig read coverage clustering suggested the presence of multiple distinct genotypes of cobionts. Contigs ascribed to *Bartonella apis* together had a total span of 11.7 Mb, almost five times longer than the reference *B. apis* genome, and formed a connected module (**Fig. 5a**). The three largest *B. apis* clusters had distinct distribution across the samples, which we suggest reflects the presence of distinct genotypes of *B. apis* with varying abundance across the samples. Similarly, contigs ascribed to *Gilliamella apicola,* the most abundant species identified in the bee microbiome, were distributed across three closely related clusters (**Fig. 5b**). Groups containing contigs from several closely related but distinct *Lactobacillus* species were identified: Firm-4 lactobacilli (clusters 25 and 40) or Firm-5 lactobacilli (clusters 16, 20, 21 and 24) (**Fig. 5c**). These *Lactobacillus* groups may represent distinct cobiont communities. The exception was cluster 21, which contained contigs assigned to a mix of Firm-5 species: this may represent a core genome component conserved between species. Cluster 29 comprised contigs assigned to *Lactobacillus kunkeei* that formed an unconnected graph component. *L. kunkeei* is thought to be an environmental rather than a gut microbiome organism. Some connected components were more complex. Cluster 32 contained contigs assigned to several prevalent honey bee cobionts, including *G. apicola, F. perrara, B. asteroides, S. alvi, B. apis, S. floricola* and *P. apium*. The co-clustering of genomic segments from multiple species is likely to reflect a strongly interacting community of organisms where the relative abundance of each is regulated homeostatically (43, 55, 59).

Some clusters had very restricted presence in the sample set. For example, cluster 3 was largely restricted to sample 4 (**Supplementary Fig. 2e**). These are likely to derive either from rare members of the honey bee cobiont community or opportunistic infections. Several clusters had little to no annotation (**Supplementary Fig. 2f**). The coverage of these contigs was also usually derived from individual

samples. They may represent novel species, or divergent or novel genomic regions of known species.

Honey bee pathogens

Known honey bee pathogens were detected in many samples. One of the largest components of clustered contigs was assigned to the trypanosomatid parasite *Lotmaria passim,* with a combined span of 16.3 Mb (**Fig. 6a**). While sequences were detected from notifiable pathogens *Melisococcus plutonious* and *Paenibacillus larvae* (European and American foulbrood), no distinct cluster was identified and the <1Mb total combined span of matched sequences was relatively minor (**Supplementary Table 2**).

Both *Nosema* species *N. apis* (**Fig. 6b**) and *N. ceranae* (**Fig. 6c**) were identified. *N. ceranae* was more prevalent (4/19 colonies *vs*. 2/19 colonies). Contigs matching the pathogen causing "chalk brood" (*Ascophaera apis*) were found in cluster 2 and were derived almost exclusively from sample 23 (**Fig. 6d**). In close proximity in the network graph was cluster 47, containing contigs assigned to the parasitic mite *V. destructor* and contigs assigned to *Apis mellifera filamentous virus* (AmFV), found in 6/19 colonies (**Fig. 6c**). The largest source of reads mapping to these contigs was sample 23, which also had a high prevalence of chalkbrood. Blobplots describing the taxonomy and cumulative span for each panel in **Fig. 6** are available in **Supplementary Fig. 2d-j**.

To validate the metagenomic hits, we employed PCR to screen our samples for *B. apis, Nosema ceranae* and *L. passim*. All samples in which we identified sequences deriving from organisms were positive by their respective PCR. However, we also identified the presence of species in additional samples not scored as positive by sequencing, suggesting that the PCR assays are more sensitive than bulk sequencing (**Supplementary Fig. 3a-c**). We also identified a small cluster containing only one contig matching to a recorded genome sequence, *Apicystis bombi*, a gregarine known to parasatise honey bees (60). To identify the exact species present, we sequenced the PCR results of custom primers against the largest contig in this cluster, in conjunction with primers encompassing the 18□S and ITS2 rDNA regions, as used by Dias *et al.* for the characterisation of novel gregarine species

(61) (**Supplementary Fig. 3d**). The contig sequence matched with various gregarine species, while the ribosomal DNA sequence confirmed the species present to be *Apicystis bombi* (**Supplementary Fig. 3e**).

Discussion

A healthy population of honey bees is crucial for the security of the ecosystem service of pollination. With the continued and sometimes unregulated global transport of *A. mellifera*, the introduction of invasive pests and parasites is a continuing threat, as is the genetic dilution or extinction of locally adapted subspecies. Here we used metagenomic analyses of nineteen honey bee colonies from around the UK to compare host genetics, examine the complexity and connectedness of the bee microbiome, and quantify disease burden.

Using the reference honey bee genome and sequence data from sixteen worker bees from each colony, we defined over five million SNVs with a relatively even distribution across all sixteen chromosomes (**Fig. 1**). We also identified likely honey bee-derived sequences not represented in the reference genome, likely because the reference is incomplete or because of variation in genomic content between honey bee populations. The island of Colonsay in Scotland is a reserve for the northern European bee, *A. m. melifera*. Given the level of bee imports into Scotland, it was therefore reassuring – and perhaps surprising – to observe that the genotypes of other colonies from around Scotland were close to that of the Colonsay sample, although distinct from samples from *A. m. mellifera* breeding programmes in England. The low heterozygosity of Scottish *A. m. mellifera* and continued survival in face of imports may reflect natural selection for *A. m. mellifera* genotypes in the colder climates and shorter foraging season of northern Europe.

The whole organism-derived sequence data was also used to explore the composition of the communities of organisms living in or on honey bees. Non-*A. Mellifera-mapping* reads were assembled to generate 160 Mb of genomic sequence from honey bee cobionts. These cobionts were biologically identified using read depth coverage, patterns of coverage across samples and best taxonomic assignment based on comparisons to known organisms. A correlation network based on per-sample read coverages of these contigs (**Fig. 2d**) did not fully match the relatedness of the source bees (**Fig. 1c**), suggesting that both environmental and host genetic components drive microbiome composition. Our limited sampling (only nineteen colonies) is not sufficient to unpick these interdependent drivers, but we

note that samples from the Scottish coast, the central belt of Scotland and from England were grouped separately. These data are congruent with previous analyses of the roles of climate and forage in determining microbiome structure of honey bees (62, 63).

In many animals, the gut microbiota form quasi-stable communities, with individual hosts harbouring somewhat predictable communities of different bacterial taxa. These different microbiome types have been associated with different gross physiological performance. In addition, changes in microbiota composition (dysbiosis) have been associated with the promotion of disease states in humans and other mammals (64, 65). Dysbiosis in honey bees may be an important correlate of bee and colony health (48, 66-68).

In the honey bee gut, bacterial numbers are highest in the rectum, followed by the ileum, mid-gut and crop (66). Lactobacilli are mainly found close to the rectum and, together with bifidobacteria, greatly outnumber other species (66). We identified several contig clusters that likely represented single *Lactobacillus* species as well as a mixed-origin cluster (**Fig. 4**). Most of these were interlinked, revealing patterns of co-occurence of individual taxa. In contrast, *L. kunkeei*, an environmental cobiont reportedly indicative of poor health (66), formed a distinct, unlinked cluster. Samples 2 and 9 were technical replicates, and both had reduced diversity, containing only *G. apicola* and *Lactobacillus* species. The reason for this is unclear, but there was no evidence of pathogenic disruption of the sampled bees.

Nosema infection has been linked to immune suppression and oxidative stress of bee hosts (69). Similarly *L. kunkeei* and *P. apium*, which are adapted to fluctuating oxygen levels predicted for the gut (70), have been associated with disease states in social bees, and negatively correlated with the amount of core commensal bacteria present (66). The microbiome from sample 23 was had a preponderance of reads mapping to the *L. kunkeei* cluster (**Supplementary Fig. 2c**), evidence of *P. apium* presence, much reduced representation of other *Lactobacillus* species, and the highest read coverage of contigs associated with the pathogens *V. destructor,* AmFV and *A. apis.* Sample 23 may be an example of pathogen-induced dysbiosis, or of invasion by pathogens of a resident microbiome disturbed by other drivers. There

was a high level of co-occurrence of different pathogens across samples, implying that colonies infected with one pathogen may be more susceptible to others. A meta-stable community may exist in the case of *Varria destructor* and AmFV (**Fig. 6c**). However, we note a recent study reported identifying 0.5 Mb of sequence from *Varroa* reference genome to be of AmFV origin (71). It is therefore possible that several of the contigs in our study matched with *Varroa destructor* are in fact of AmFV origin.

Several distinct contig clusters were assigned to *G. apicola* and *B. apis* suggesting the existence of genetically distinct subtypes of these highly prevalent bacteria. (**Fig. 5a,b**). *G. apicola* has a high diversity of accessory genes, associated with adaptation to different *A. mellifera* ecological niches (72, 73). Increased relative abundance of *G. apicola* has been associated with dysbiosis and host deficiencies (66). Similarly, extreme displacement of *S. alvi* by *F. perrara* and *G. apicola* (and to a lesser extent by the opportunists *P. apium* and *L. kunkeei*) has been strongly associated with reduced bacterial biofilm function and host tissue disruption by scab-inducing *F. perrara* (48, 68), leading to poor host development and early mortality. Blooms of *B. apis* have also been associated with poor health. This species exploits stressed, young, and old bees, showing sporadic abundance in whole guts of newly emerged workers (58) and occurring uniformly across putatively dysbiotic foragers (56). In support of this theory, samples from our study with the highest coverage of *G. apicola* and *B. apis* contigs also contained reads from pathogens such as *L. passim* or *Nosema* species. Significant positive correlation has been reported between infection levels of these parasites (74).

Our novel use of correlation networks (**Fig. 3**) to organise contigs based on their relative abundance across samples partitioned 65% of them into clusters of sequences corresponding to individual species and distinct micro-communities. Some sample-specific clusters, such as clusters 3 and 32, contained several core microbiome taxa. This may be a reflection of substrate specialisation based on host foraging (75). However, several sample specific clusters contained contigs that had no informative taxonomic annotation, potentially revealing uncharacterised species. We identified a cluster of unclassified contigs derived from a gregarine, with closest match to *Apicystis bombi*. The accuracy of our metagenomic analyses was

388 confirmed by PCR and ribosomal DNA primers verified the species as *Apicystis*
389 *bombi*. This is further evidence that managed honey bees can act as a reservoir for
390 wild pollinator pathogens (60); through increased understanding of honey bee
391 molecular ecology and preventing disease transmission, we can indirectly improve
392 wild pollinator health (76). To our knowledge *Lotmaria passim* had not been
393 previously identified in the UK. Its presence was confirmed for the first time in our
394 study using the primers designed by Stevanovic *et al*. (77), further validating our
395 sequencing inference.

396 A whole-organism metagenomics approach has allowed us to describe the
397 complexity of host-microbiome biology of UK honey bees. Using pooled samples we
398 have demonstrated the power of this approach in dual characterisation of the
399 genotypic diversity of the honey bee and the genomic diversity of its cobionts.
400 Correlation networks are powerful analytic tools that allowed us to cluster the
401 sequence data to reveal interacting networks of bacterial and eukaryotic microbiota,
402 in addition to classifying novel genomic sequences. As with the human and other
403 animal microbiome projects, the precision of these analyses improves with additional
404 data, permitting definition (and ultimately whole genome assembly) of novel
405 genotypes of cobionts. Complementation of cheap short read data with low-coverage
406 long-read data from isolated gut contents enhances the contiguity of assemblies and
407 the functional inferences that can be derived them. This study highlights the potential
408 to use this approach in routine screening, breeding programmes and horizon
409 scanning for emerging pathogens.

410

Methods

Samples

Nineteen samples of honey bee (each comprising sixteen workers collected from a single colony) were obtained from beekeepers in Scotland and England, with the help of Science and Advice for Scottish Agriculture (SASA) and Fera Science Ltd. Wings, legs and heads were removed before homogenizing the remainder of the bees (thorax and abdomen) in 2% CTAB buffer (100 mM Tris-HCl pH 8.0, 1.4 M NaCl, 20 mM EDTA pH 8.0, 2% hexadecyltrimethylammonium bromide, 0.2% 2-mercaptoethanol). Samples were incubated at 60^{o}C with proteinase K (54 ng/µl) for 16 h before incubating with RNaseA (2.7 ng/µl) at 37^{o}C for 1 h. After two chloroform:isoamyl alcohol (24:1) extractions, samples were ethanol precipitated, washed three times in 70% ethanol and resuspended in 0.1 TE. All genomic DNA samples were analysed for quantity (Qubit dsDNA HS Assay Kit, Thermo Fisher Scientific, Waltham, MA, USA), purity (Nanodrop, Thermo Fisher Scientific, Waltham, MA, USA) and quality (TapeStation, Agilent Technologies, Santa Clara, CA, USA).

Sequencing

All sequencing was performed by Edinburgh Genomics. DNAs were prepared for whole genome sequencing using the TruSeq DNA PCR-free gel free library kit (Illumina, Cambridge, UK) and, for eight samples, using the TruSeq DNA Nano gel free library kits (Illumina). For comparison, both types of libraries were prepared for four samples. 125 base paired-end sequencing was performed on an Illumina HiSeq 2500. Four samples were sequenced at 50X coverage, eight at 25X (including repeat sequencing of the four 50X samples) and twelve at 17X coverage. Data were screened for quality using FastQC v0.11.2 (Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc), and trimmed of low quality regions and adapters using Trimmomatic v0.35 (78) with parameters 'TRAILING:20 SLIDINGWINDOW:4:20 MINLEN:100.' These parameters remove bases from the end of a read if they are below a Phred score of 20, clip the read if the average Phred score within a 4 base sliding window advanced from the 5' end

441 falls below 20, and specify a minimum read length of 100 bases (the parameters
442 used for all informatics analyses are also detailed in **Supplementary Table 3**).

443 ## Variant calling on honey bee

444 Reads were aligned to the reference *A. mellifera* genome, Amel_4.5 (INSDC
445 assembly GCA_000002195.1) using BWA-MEM v0.7.8 (79) with parameters -R and
446 -M. Output files were merged and duplicates marked using Picard Tools v2.1.1 to
447 create one BAM file per sample. This was filtered using SAMtools view v1.3 (80) to
448 retain only the highest confidence alignments using the parameters -q 20 (to remove
449 alignments with a Phred score <20) and -F 12 (to remove all reads that are not
450 mapped and whose mate is not mapped).

451 Variants were called using GATK v3.5 in accordance with GATK best practice
452 recommendations (81, 82). Local realignments were performed and base quality
453 scores recalibrated using bee SNVs from dbSNP (83) build ID 140
454 (ftp://ftp.ncbi.nlm.nih.gov/snp/organisms/bee_7460/VCF/, downloaded 1st January
455 2016). GATK HaplotypeCaller was used with parameters emitRefConfidence, -
456 GVCF variant index type – LINEAR, variant index parameter -128000, stand emit
457 conf – 30, stand call conf - 30. The resulting VCFs, one per sample, were merged to
458 create a single gVCF file using GATK GenotypeGVCFs to allow variants to be called
459 on all samples simultaneously. Variant quality score recalibration was performed on
460 this file using GATK VariantRecalibrator with parameters badLodCutoff – 3, -an QD,
461 -an MQ, -an MQRankSum, -an ReadPosRankSum, -an FS, -an DP (specifying the
462 above dbSNP data as both the truth set [prior=15.0] and training set [prior=12.0]). To
463 identify any effect these variants may have upon protein-coding genes in the
464 reference annotation, we used SNPeff v4.2 (84). A total of 5,302,201 variants were
465 identified across the 19 samples.

466 An Identity By State (IBS) analysis was performed using the R/Bioconductor
467 package, SNPRelate (85). Briefly, the minor allele frequency and missing rate for
468 each SNV was calculated over all the samples. The values of the resultant IBS
469 matrix ranged from zero to one. Using this matrix, we constructed a network
470 correlation graph for all of the samples, using the network analysis tool Graphia
471 Professional (Kajeka Ltd., Edinburgh, UK), where each node represented a sample,

and edges between nodes represented a correlation above the defined threshold between those samples.

De novo assembly and analysis of non-honey bee data

De novo assembly was performed on all of the reads which did not map to the *Apis mellifera* reference genome using SPAdes v3.8.1 (86). The resulting contigs were filtered by length (> 1kb) and coverage (> 2). BWA-MEM (79) was used to identify and remove reads mapping to these contigs and *de novo* assembly was performed on the remaining reads. This process was repeated for a total of five iterations. Input reads from each sample were mapped to each contig using BWA-MEM and base coverage/contig was calculated. Contigs with a cumulative base coverage from all samples less than half the SPAdes overall coverage were discarded. Using BLAST (87), contigs were compared to a set of custom databases: 1. HB_Bar_v2016.1 (53); 2. HB_Mop_v2016.1 (53); 3. nucleotide sequences of core microbiome species identified from literature (43, 44, 55, 73); 4. protein sequences of these species (43, 44, 55, 73); 5. NCBI nt (88); 6. UniProt Reference Proteomes (89) using BLAST (87) and Diamond (90). Files of all six sequence similarity searches were provided as input to BlobTools in the listed order under the tax-rule 'bestsumorder', *i.e.* a contig is assigned the NCBI taxid of the taxon providing the best scoring hits within a given file, as long as it has not been allocated a NCBI taxid in a previous file.. BlobTools was used to visualise the coverage, GC% and best BLAST similarity match of the assembly, and to build a table of base coverage of contigs in each sample together with their taxonomic annotation. A network graph was constructed using *r* value of 0.99 comparing samples to each other based on correlations between their overall microbiome content as well as contig coverage across the dataset. This follows the approach used to compare gene expression values in transcriptomics data (91).

Population genetics analyses

SNVs were filtered using Plink v1.9; (92) to remove those not mapped to the autosomes, those having low genotyping call rate (<0.9), those with low minor allele frequency (<0.1), and those with pairwise linkage disequilibrium $r^2 > 0.1$ (for SNVs in 50 kb windows with a 10 kb step). The resulting 58,354 SNVs were submitted to unsupervised analyses in ADMIXTURE (93) for $1 \leq K \leq 5$ genetic backgrounds. To

503 explore consequence of analysing genotypes from pooled DNA, individual genotypes
504 simulated for 10 individuals per sampling location for each SNV were subjected to
505 ADMIXTURE analysis. Briefly, for each SNV the allele frequency observed in a
506 pooled sample was calculated from the read counts for each allele, and used to
507 simulate ten genotypes assuming Hardy-Weinberg equilibrium. The efficacy of this
508 process was tested using data from Harpur *et al.* (94), details of which are provided
509 in the supplementary information (**Supplementary Table 3**). A distance matrix from
510 the pooled DNA genotypes used in ADMIXTURE analyses was generated with Plink
511 and analysed using the R package netview (95) (https://github.com/esteinig/netview),
512 which analyses genetic structure using mutual k-nearest neighbour (kNN) graphs.
513 Graphs were created assuming $2 \leq k \leq 20$ nearest neighbours.

514 *Primer design for identification of cobionts using PCR*

515 Custom primers were designed against the longest contigs we generated matching
516 *Bartonella apis* (Bartonella_Fw 5'-CAGCAGCGCTTATTCCGTTC-3', Bartonella_Rv
517 5'-AGTCACGAGCAACAATCGGT-3') and the Gregarine species (Gregarine_F 5'-
518 GACCACCGTCCTGCTGTTTA-3', Gregarine_R 5'-GAGGTATCGGGTGCCATGA-
519 3'). Primers were run through NCBI BLAST to confirm specificity (87). *Apicystis*
520 *bombi* specific primers were used as described in Dias *et al.* (61). Specific primers
521 against *Nosema ceranae* were used as described by Chen *et al.* (96) and *Lotmaria*
522 *passim* specific primers were used as described by Stevanovic *et al.* (77).

523 *Rarefaction analysis of microbiome sampling*

524 "Mean species richness" was calculated using the R package 'vegan' (97) for each
525 sample at each of the sequencing depths used. Assembled contigs were analysed
526 against the SILVA rDNA (16S and 18S) databases (98) instead of the NCBI nt
527 database to assess species composition. Each contig identified as being from a
528 unique species was counted as one "count" or incidence of discovering that species
529 in the sample.

530

Figures

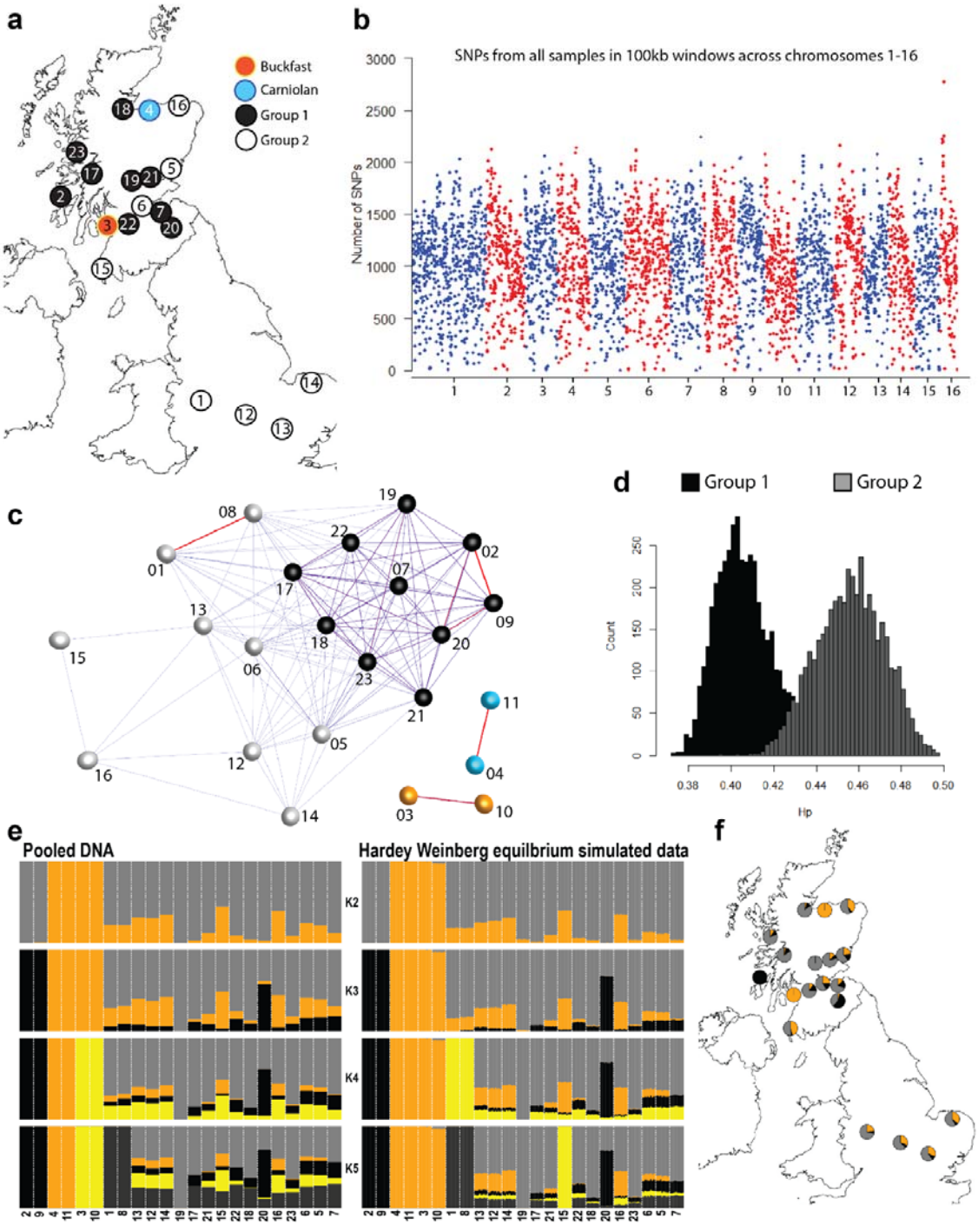


Figure 1: *Apis mellifera* diversity. (A) A map of the UK with the location of colonies sampled. **(B)** The number of SNVs from all samples presented across *A. mellifera* chromosomes 1 to 16 in 100 kb consecutive windows. **(C)** A network based on the identity by state (IBS) similarity score of sample variants identifying Groups 1 and 2 in the major cluster. This includes sequencing duplicates (01-04). **(D)** The heterozygosity level across consecutive window of size 100 kb comparing groups 1 and 2 identified from the network graph. **(E)** ADMIXTURE analyses of pooled DNA (left) and genotypes simulated assuming Hardy Weinberg equilibrium (right). **(F) M**ap of sampling locations indicating ADMIXTURE results at K = 3.

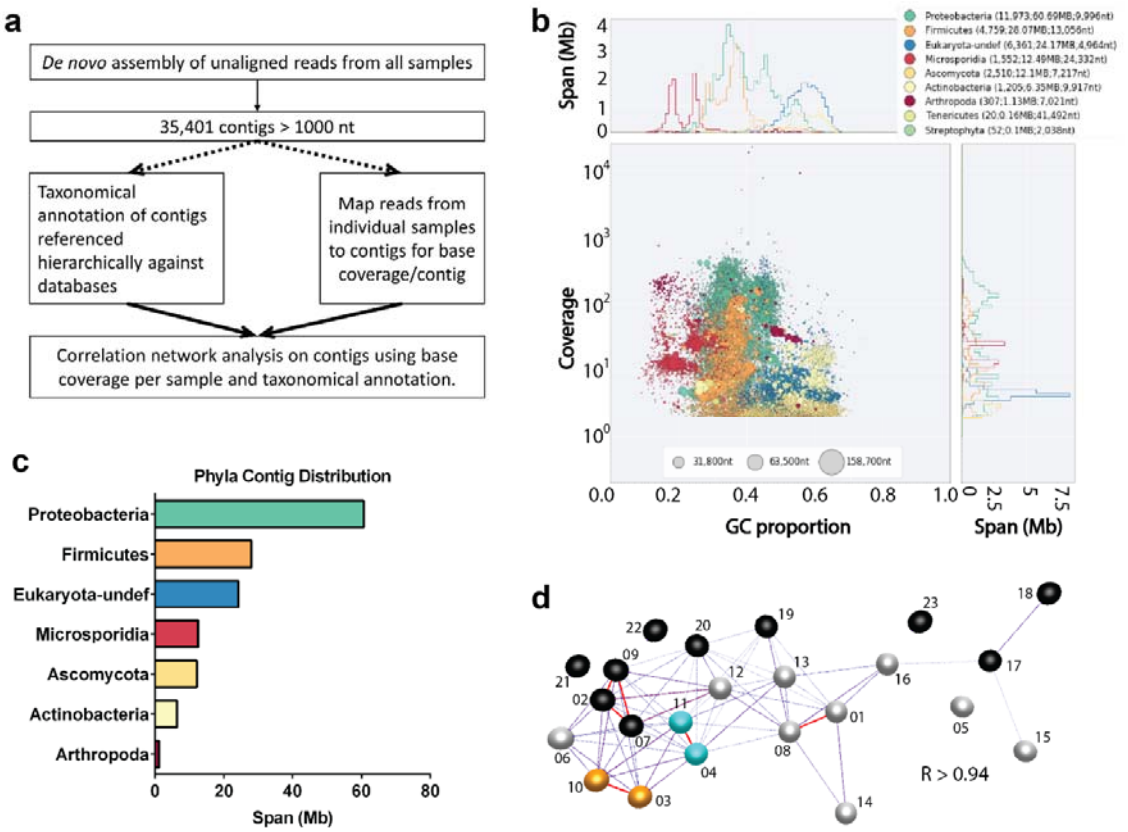


Figure 2: Metagenomics of *Apis mellifera.* (A) A flow diagram of the microbiome analysis using reads which did not align to the *Apis mellifera* reference genome. **(B)** A blobplot generated from contigs using unaligned reads from all samples. Contigs are plotted based on their GC content (x-axis) and coverage (y-axis), scaled by span, and coloured by their phylum assignation. **(C)** The span of *de novo* assembled contigs which were assigned to given phyla is displayed for the 12 most abundant phyla across all samples. **(D)** A network based on the coverage/contig from each sample representing microbiome composition/unaligned reads.

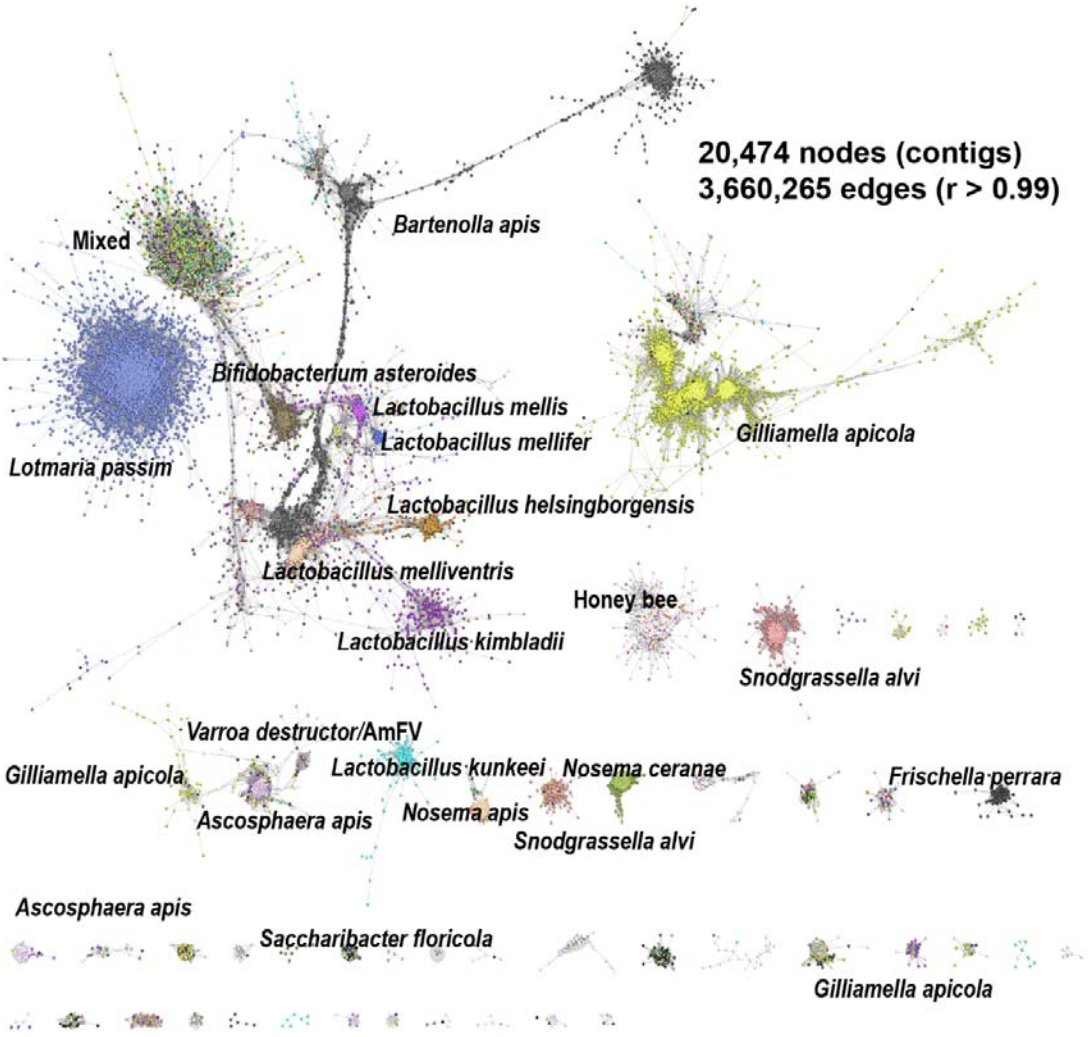


Figure 3: Correlation network analysis of microbiome contigs. Each node represents an individual contig and edges are defined based on the abundance profile of the number of reads mapping to the contig across individual samples. Contigs (nodes) are connected if the Pearson correlation between two contigs abundance profile was $r > 0.99$. Each contig is coloured according to species ID, white nodes represent contigs for which no significant sequence match was found.

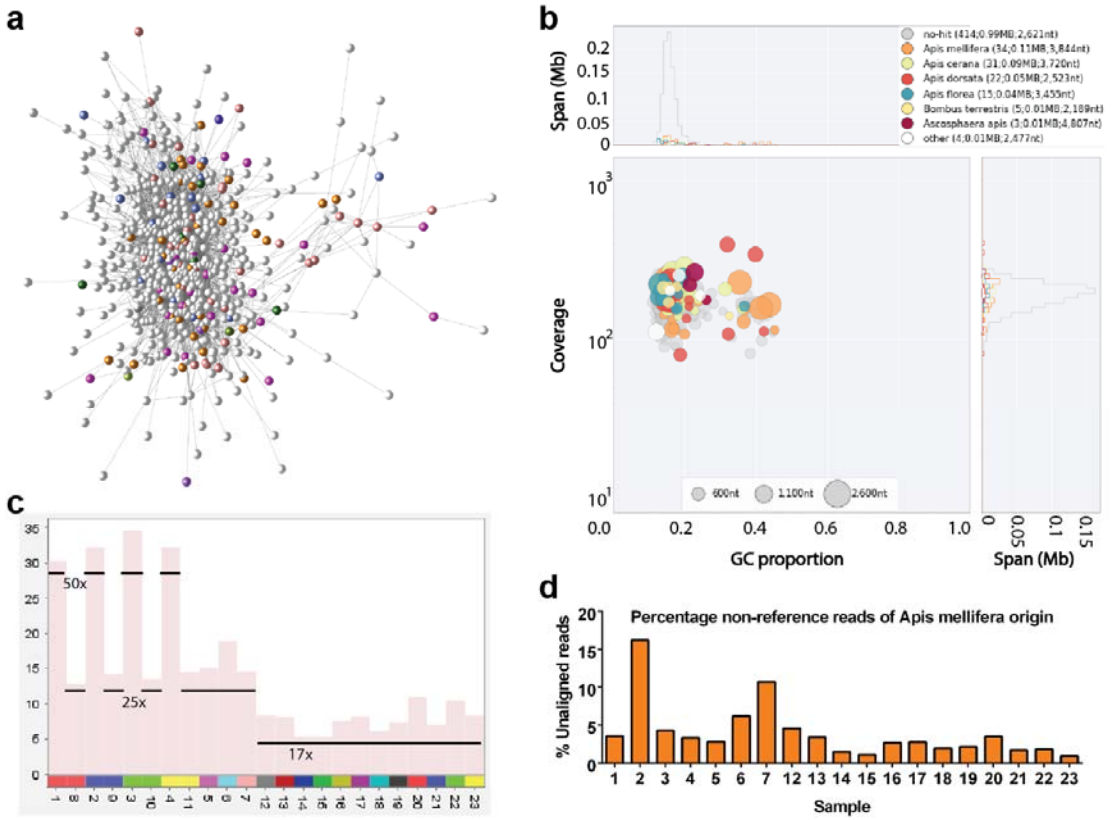


Figure 4: Putative *Apis mellifera* contigs. (A) Network component of contigs which did not match the reference bee genome and are unassigned (white) or match a non-reference species of bee (coloured). **(B)** Blobplot of these contigs (as in Figure 2)**. (C)** Mean base coverage per contig (y-axis) for each sample (x-axis) for the contigs in A. The sequencing depth (reference genome coverage) per sample is shown, showing that the number of reads mapping to these contigs is in direct proportional to the depth of sequencing. **(D)** A graph displaying the percentage of unaligned reads putatively identified as *Apis mellifera* from each sample.

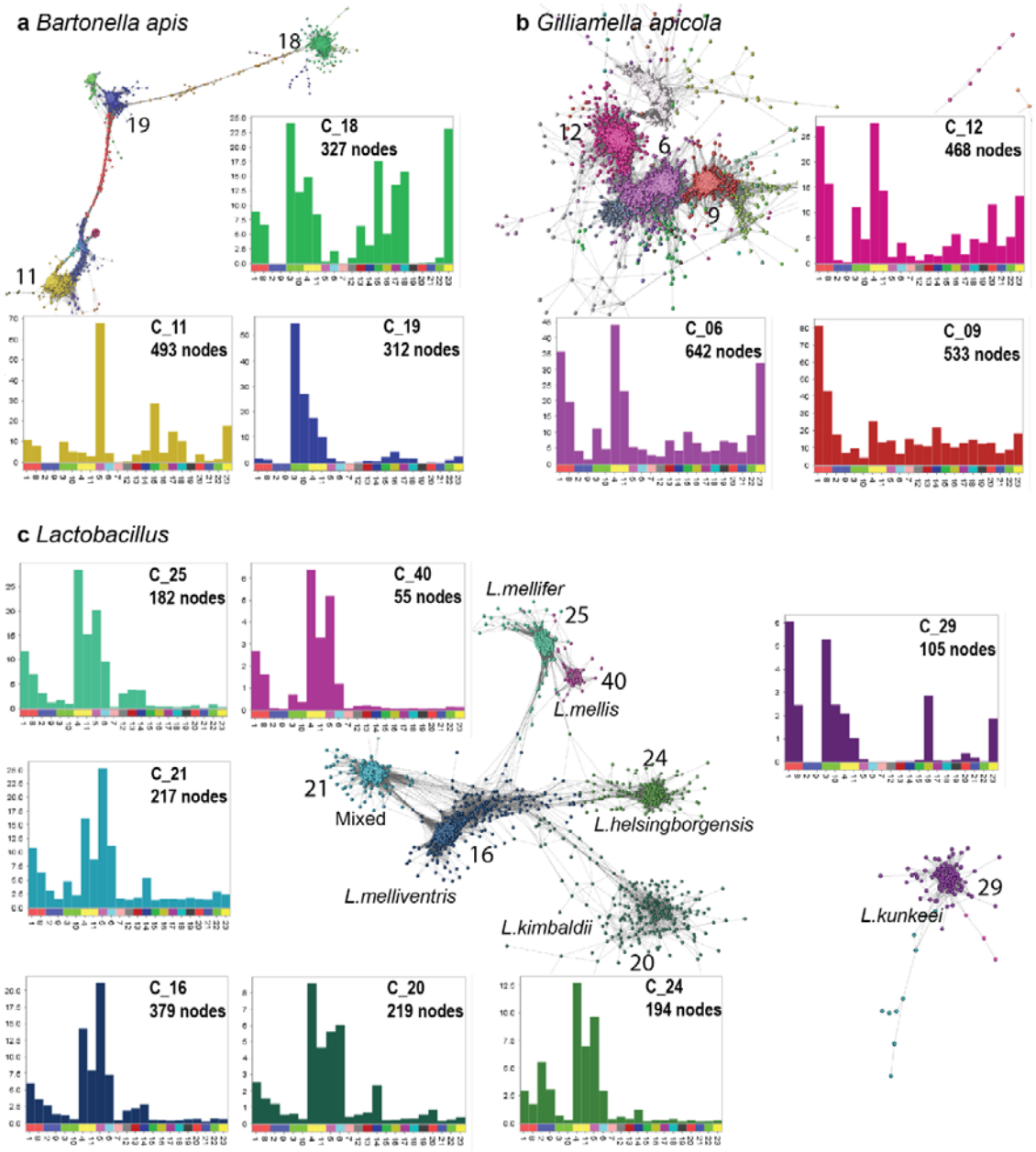


Figure 5: Communities of honey bee cobionts. Sub-networks of contig clusters from Figure 3 coloured by cluster. Histograms show the mean base coverage per contig (y-axis) for each sample (x-axis). **(A)** *Bartonella apis*, **(B)** *Gilliamella apicola* and **(C)** several *Lactobacillus* species. Blobplots describing the taxonomy and cumulative span for each of these panels are presented in **Supplementary Figure 1A-C**.

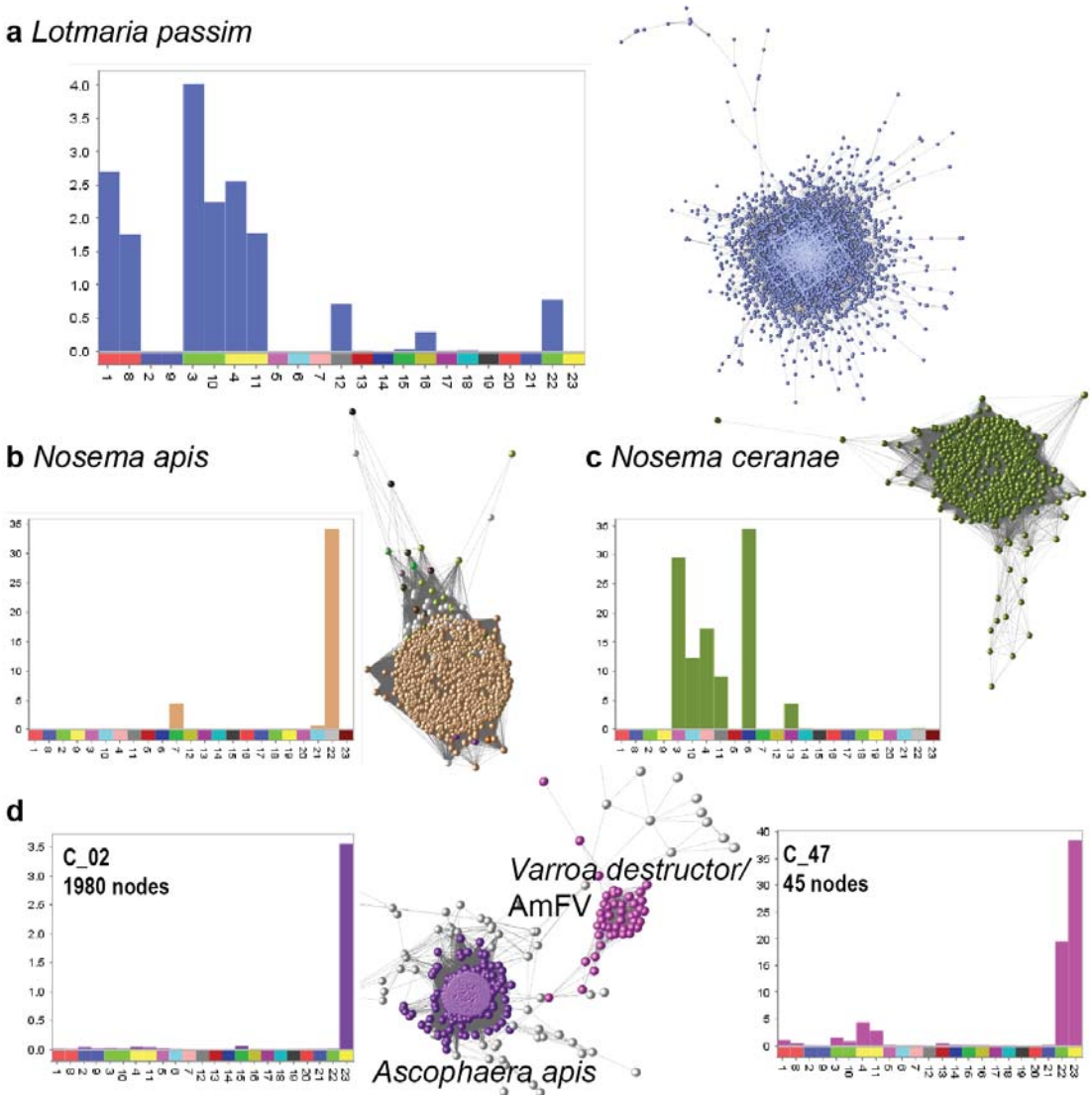
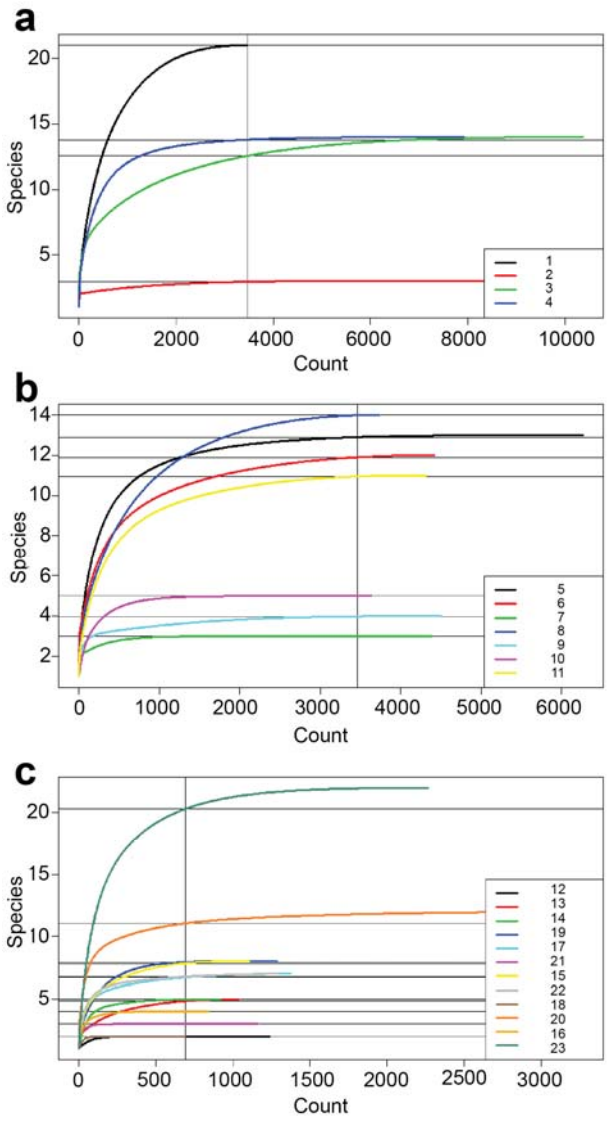
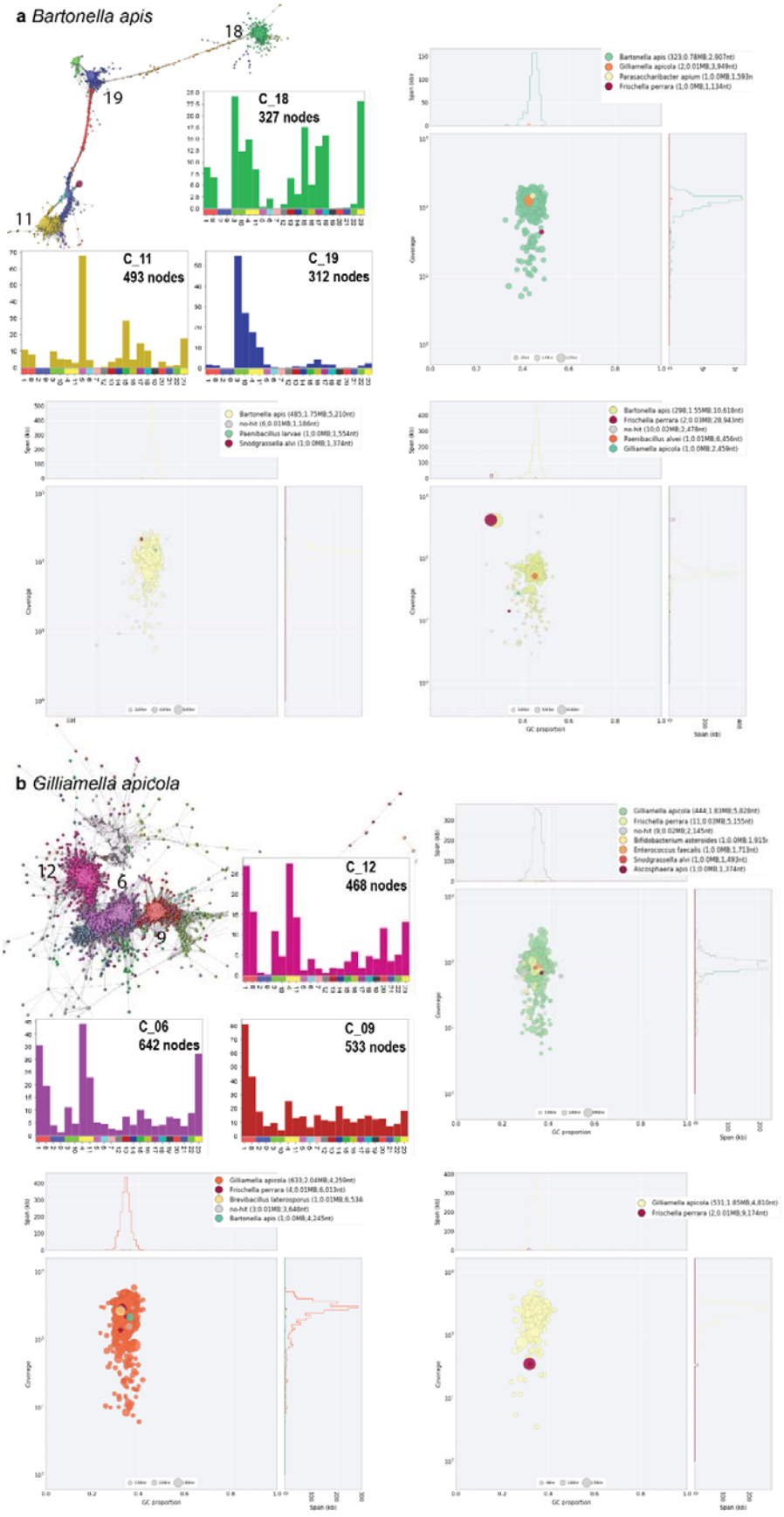
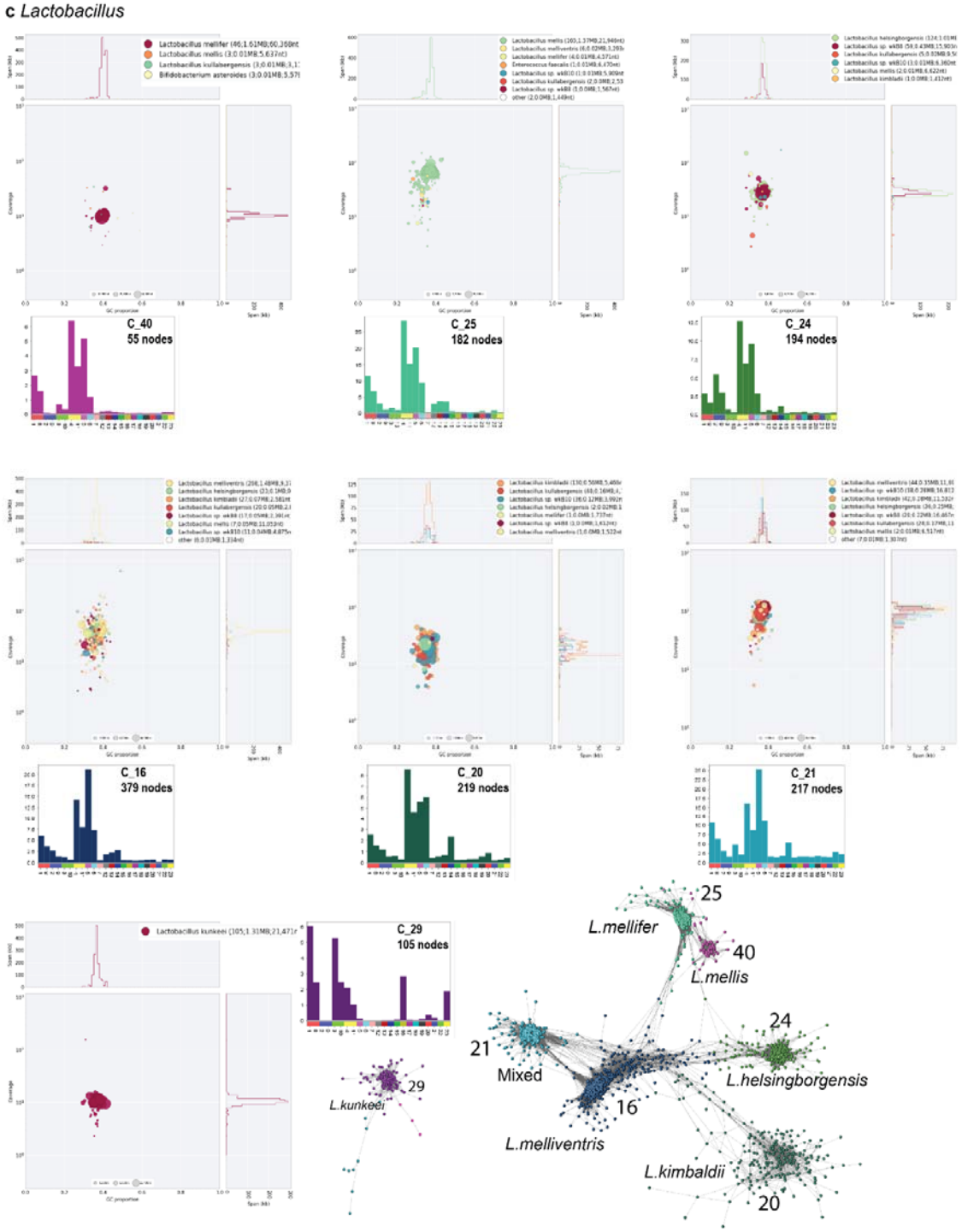


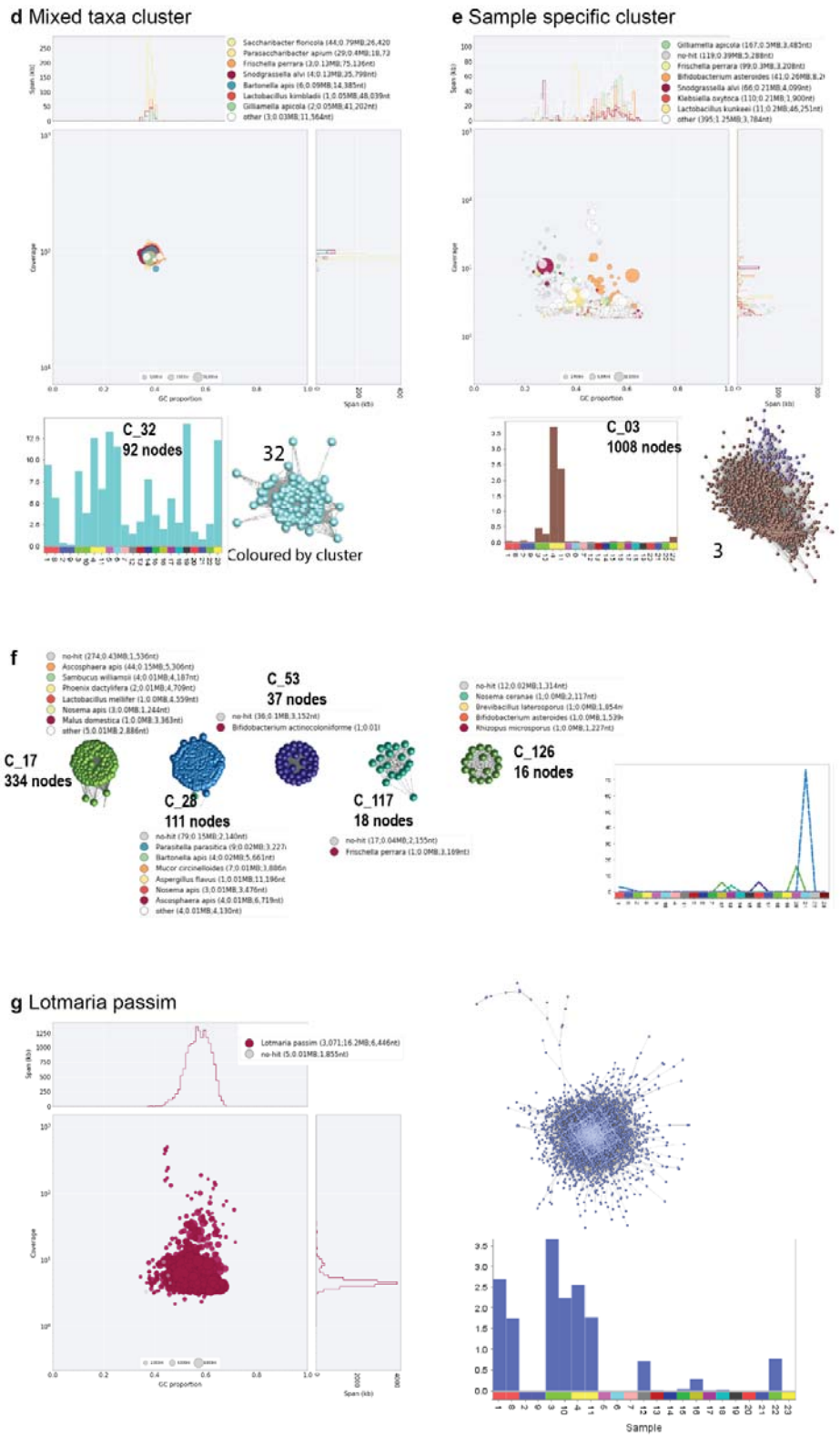
Figure 6: Disease associated components. Clusters associated with honey bee cobionts including mean base coverage per contig (y-axis) for each sample (x-axis). **(A)** *Lotmaria passim*, **(B)** *Nosema apis*, **(C)** *Nosema ceranae* and **(D)** a community of species including *Ascophaera apis* (associated with chalkbrood), *Varroa destructor* and *Apis mellifera filamentous virus* (AmFV). Blobplots describing the taxonomy and cumulative span for each panel are presented in Supplementary Figure 1D-J.

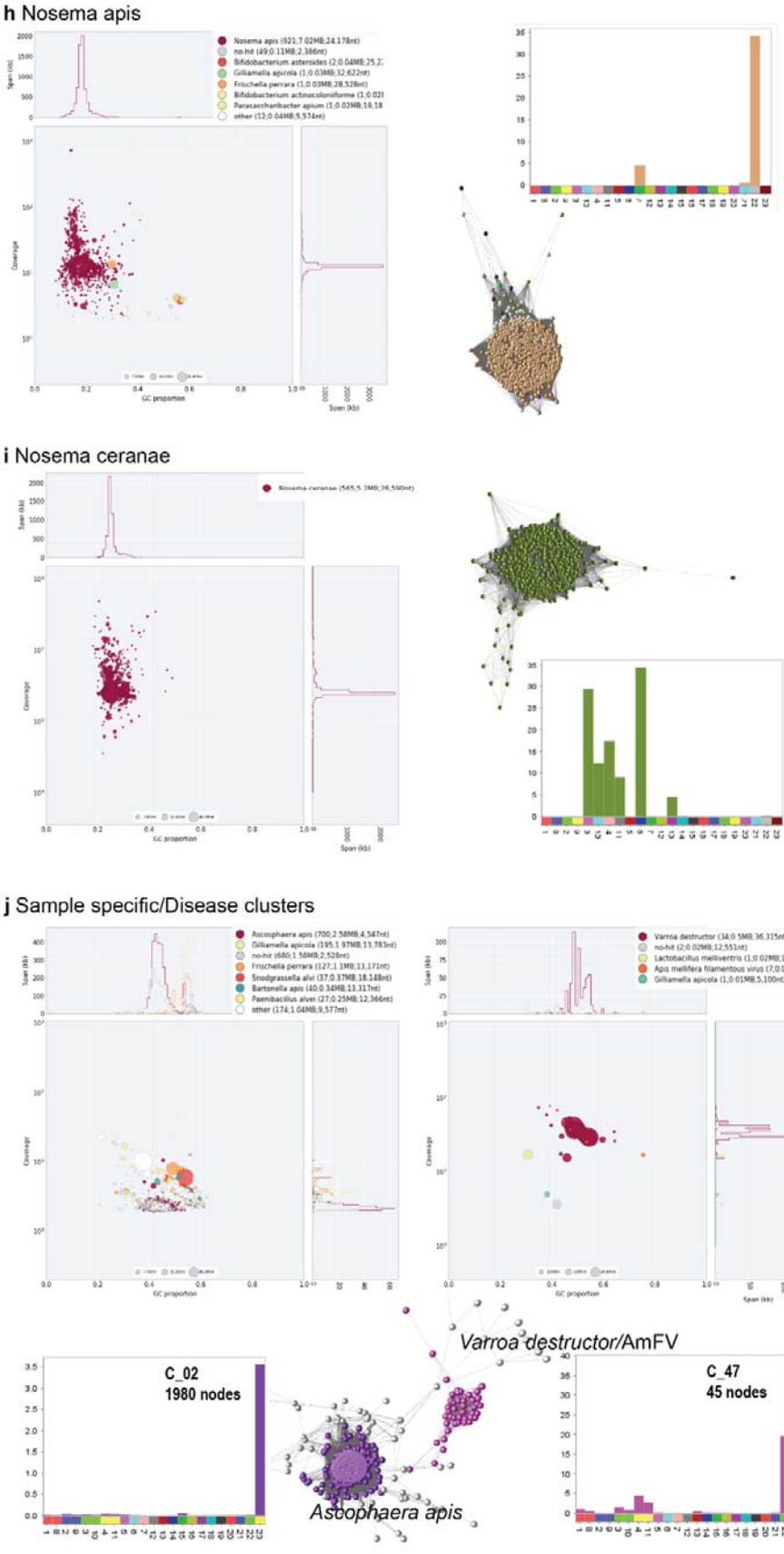


Supplemental Fig. 1: Rarefaction plot of micro-organisms identified. Mean species richness is displayed for all samples at each of the sequencing depths analysed: 50X reference genome coverage (A), 25X reference genome coverage (B) and 17X reference genome coverage (C). Each count on the x-axis represents a contig matching a unique species in the rDNA SILVA database while total number of species is displayed on the y-axis.



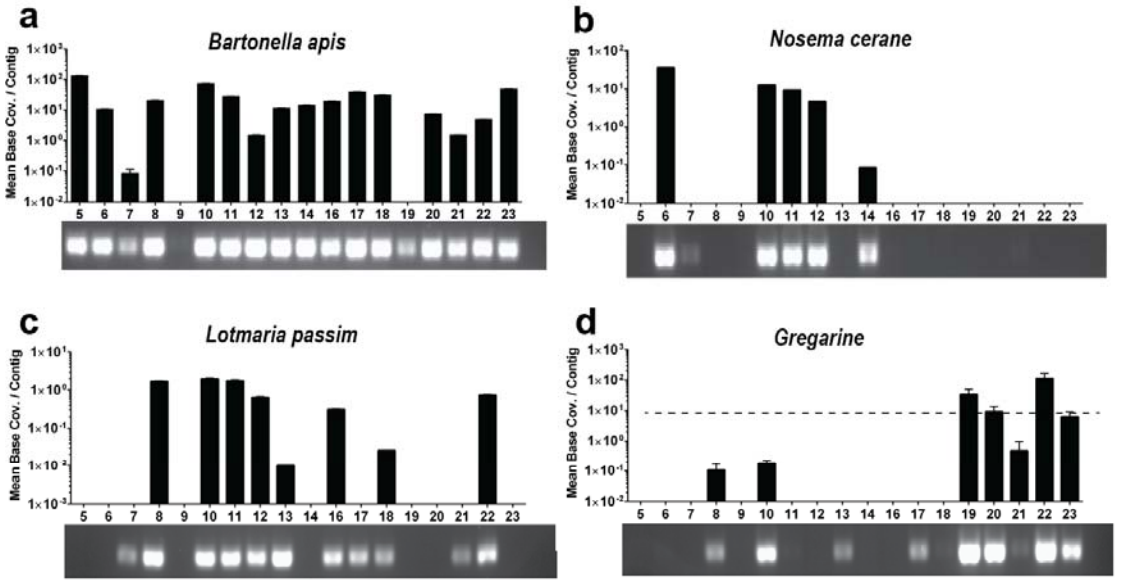






Supplementary Figure 2 Taxonomic summary of contig clusters

Clusters associated with honey bee cobionts including mean base coverage per contig (y axis) for each sample (x axis) and associated blobplot for **(A)** *Bartonella apis*, **(B)** *Gilliamella apicola* and **(C)** various *Lactobacillus* species, **(D)** a cluster of heterogeneous core taxa, **(E)** a cluster of contigs specific to sample 3, **(F)** clusters of contigs with poor annotation, **(G)** *Lotmaria passim*, **(H)** *Nosema apis*, **(I)** *Nosema ceranae* and **(J)** a community of species including *Ascophaera apis* (associated with chalkbrood), *Varroa destructor* and *Apis mellifera filamentous virus*.



e

Description	Accession	Max score	Query cover	E value	Identity
Gregarine contig					
Ascogregarina taiwanensis 18S ribosomal RNA gene, partial sequence; and internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2, 26S ribosomal RNA and 5S ribosomal RNA genes, complete sequence	EF666482.1	1454	80%	0	88%
Neogregarinorida sp. OPPPC1 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA	AB748927.1	1378	75%	0	90%
Apicystis bombi partial ssu rRNA gene for small subunit ribosomal RNA, isolate Irw1	FN546182.1	2084	28%	0	100%
Apicystis sp. isolate Bpas1 18S small subunit ribosomal RNA gene, partial sequence	MF998086.1	1820	29%	0	95%
Mattesia sp. SV-2003 external transcribed spacer and 18S ribosomal RNA gene, partial sequence	AY334569.1	1796	30%	0	93%
Gregarine rDNA					
Apicystis bombi isolate NPaw1 small subunit ribosomal RNA gene, partial sequence	HQ619890.1	747	77%	0	99%
Apicystis bombi partial ssu rRNA gene for small subunit ribosomal RNA, isolate Irw1	FN546182.1	688	71%	0	99%
Apicystis bombi gene for small subunit ribosomal RNA, partial sequence	AB738024.1	686	70%	0	99%
Mattesia sp. SV-2003 external transcribed spacer and 18S ribosomal RNA gene, partial sequence	AY334569.1	656	86%	0	92%
Apicystis sp. isolate Bpas1 18S small subunit ribosomal RNA gene, partial sequence	MF998086.1	616	77%	3.00E-172	94%

Supplementary Figure 3: PCR based identification of known and putative *Apis mellifera* cobionts. Mean base coverage/contig (y axis) matched with the taxa indicated for each sample (x axis) against PCR bands for **(a)** *Batonella apis*, **(b)** *Nosema ceranae,* **(c)** *Lotmaria passim* and an **(d)** a putative gregarine derived contig from Cluster 239 **(e)** BLAST results of PCR product sequences from (d) and rDNA.

Sample ID	Vice county and postcode	Collection organized by	Coverage	% mapped to reference	Colony Information
1	Shropshire (SY8)	Roslin Institute	50X	89.4	A. m. mellifera breeding project
2	Fifeshire (KY13)	Roslin Institute	50X	97	A. m. mellifera (Colonsay Queen)
3	Ayrshire (KA23)	Roslin Institute	50X	92.1	Buckfast
4	Moray (IV36)	Roslin Institute	50X	91	Carniolan
5	Angus (DD3)	SASA	25X	88	Scottish population
6	West Lothian (EH48)	SASA	25X	93.3	Scottish population
7	Midlothian (EH25)	Roslin Institute	25X	95.9	Scottish population
8*	Shropshire (SY8)	Roslin Institute	25X	88.8	A. m. mellifera breeding project
9*	Fifeshire (KY13)	Roslin Institute	25X	97.3	A. m. mellifera (Colonsay Queen)
10*	Ayrshire (KA23)	Roslin Institute	25X	91.2	Buckfast
11*	Moray (IV36)	Roslin Institute	25X	89.9	Carniolan
12	Warwickshire	Fera	17X	91.1	A. m. mellifera (England)
13	Cambridgeshire	Fera	17X	93.7	A. m. mellifera (England)
14	Norfolk	Fera	17X	86.2	A. m. mellifera (England)
15	Wigtownshire (DG9)	SASA	17X	82.2	Scottish population
16	Banffshire (AB45)	SASA	17X	89.4	Scottish population
17	Argyllshire (PA38)	SASA	17X	90.5	Scottish population
18	East Ross and Cromarty (IV8)	SASA	17X	89	Scottish population
19	Mid Perthsire (PH6)	SASA	17X	88	Scottish population
20	Selkirkshire (TD7)	SASA	17X	92.1	Scottish population
21	East Perthshire (PH1)	SASA	17X	87	Scottish population
22	Lanarkshire (G74)	SASA	17X	87.2	Scottish population
23	West Inverness-shire (PH41)	SASA	17X	77.1	Scottish population

*Technical replicates of Samples 1-4.

Supplementary Table 1. Samples 1 to 4 were sequenced at depths of both 50 and 25 times reference genome coverage. All other samples were sequenced once at the depth indicated.

Species	Phylum	Span	Score	Count
Gilliamella apicola	Proteobacteria	2.12E+07	2.15E+09	4828
Snodgrassella alvi	Proteobacteria	7.68E+06	1.29E+09	1816
Frischella perrara	Proteobacteria	8.18E+06	7.30E+08	1200
Bartonella apis	Proteobacteria	1.17E+07	6.23E+06	2694
Serratia fonticola	Proteobacteria	3.64E+05	4.18E+05	177
Klebsiella oxytoca	Proteobacteria	2.52E+05	3.57E+05	139
Parasaccharibacter apium	Proteobacteria	2.39E+06	1.89E+05	251
Hafnia alvei	Proteobacteria	1.77E+05	1.87E+05	65
Acinetobacter johnsonii	Proteobacteria	1.28E+05	1.50E+05	65
Klebsiella michiganensis	Proteobacteria	9.46E+04	1.13E+05	46
Saccharibacter floricola	Proteobacteria	2.73E+06	9.28E+04	208
Lotmaria passim	Trypanosomatida	2.40E+07	1.33E+08	6340
Lactobacillus kimbladii	Firmicutes	2.15E+06	4.16E+08	524
Lactobacillus kullabergensis	Firmicutes	1.67E+06	3.41E+08	503
Lactobacillus sp. wkB8	Firmicutes	9.47E+05	1.82E+08	183
Lactobacillus melliventris	Firmicutes	2.88E+06	1.37E+08	574
Lactobacillus helsingborgensis	Firmicutes	1.88E+06	1.14E+08	318
Lactobacillus kunkeei	Firmicutes	2.51E+06	1.09E+08	421
Lactobacillus mellis	Firmicutes	3.31E+06	1.07E+08	436
Lactobacillus mellifer	Firmicutes	2.33E+06	3.90E+07	176
Lactobacillus sp. wkB10	Firmicutes	1.26E+06	2.69E+07	346
Paenibacillus larvae	Firmicutes	6.21E+05	2.94E+06	127
Melissococcus plutonius	Firmicutes	3.71E+05	2.18E+06	25
Nosema apis	Microsporidia	7.05E+06	1.77E+07	932
Nosema ceranae	Microsporidia	5.30E+06	8.41E+06	598
Bifidobacterium asteroides	Actinobacteria	5.20E+06	8.72E+08	1057
Aspergillus flavus	Ascomycota	4.81E+04	6.32E+06	11
Ascosphaera apis	Ascomycota	2.92E+06	3.61E+05	805
Apis mellifera	Arthropoda	1.74E+05	5.48E+04	83
Apis cerana	Arthropoda	1.40E+05	3.61E+04	63
Varroa destructor	Arthropoda	5.37E+05	3.02E+04	51
Apis dorsata	Arthropoda	8.50E+04	2.04E+04	42
Apis florea	Arthropoda	8.06E+04	1.68E+04	35
Sambucus williamsii	Streptophyta	2.94E+04	3.70E+04	13
Trifolium repens	Streptophyta	3.26E+04	2.22E+04	19
Spiroplasma apis	Tenericutes	3.98E+04	1.76E+03	10
Apis mellifera filamentous virus	Viruses-undef	1.67E+04	1.40E+04	12
Apicystis bombi	Apicomplexa	8.33E+03	5.89E+02	3
no-hit	no-hit	6.22E+06	0.00E+00	3264
undef	undef	5.25E+06	5.44E+05	1864

Supplementary Table 2 The top 34 species ID hits from *de novo* assembled non-reference matching reads.

Tool(s) used	Application	Parameters	Source
FastQC v0.11.2	Quality control of sequencing reads		http://www.bioinformatics.babraham.ac.uk/projects/fastqc
Trimmomatic v0.35	Trimming reads of low quality regions and adapters	'TRAILING:20 SLIDINGWINDOW:4:20 MINLEN:100.	(78)
BWA-MEM v0.7.8	Reference assembly mapping of reads	-R and -M	
Picard Tools v2.1.1	Merging of read files and marking of duplicates		http://broadinstitute.github.io/picard/
SAMtools view v1.3	Remove low quality alignments	-q 20	
GATK v3.5 Haplotype Caller	Variant calling	--emitRefConfidence GVCF --variant_index_type LINEAR --variant_index_parameter 128000 -stand_emit_conf 30 -stand_call_conf 30	
GATK v3.5 VariantRecalibrator	Variant quality score recalibration	-badLodCutoff -3 -an QD -an MQ -an MQRankSum -an ReadPosRankSum -an FS -an DP, dbSNP data as both the truth set (prior=15.0) and training set (prior=12.0)	
SNPeff v4.2	Identify SNVs affecting protein coding regions		
SPAdes v3.8.1	*De novo* assembly of non-*Apis mellifera* mapping reads	--careful -t 40	
blastn v2.6.0+	Assigning species identification to contigs from *de novo* assembly	-task megablast -outfmt '6 qseqid staxids bitscore std sscinames sskingdoms stitle' -culling_limit 5 -num_threads 32 -evalue 1e-25	
Diamond v0.9.5 blastx	Assigning species identification to contigs from *de novo* assembly	--max-hsps 1 --sensitive --evalue 1e-25 --max-target-seqs 1 --outfmt 6	
Blobtools	Species assignation and visualisation of contigs		

Supplementary Table 3 Bioinformatics parameters used.

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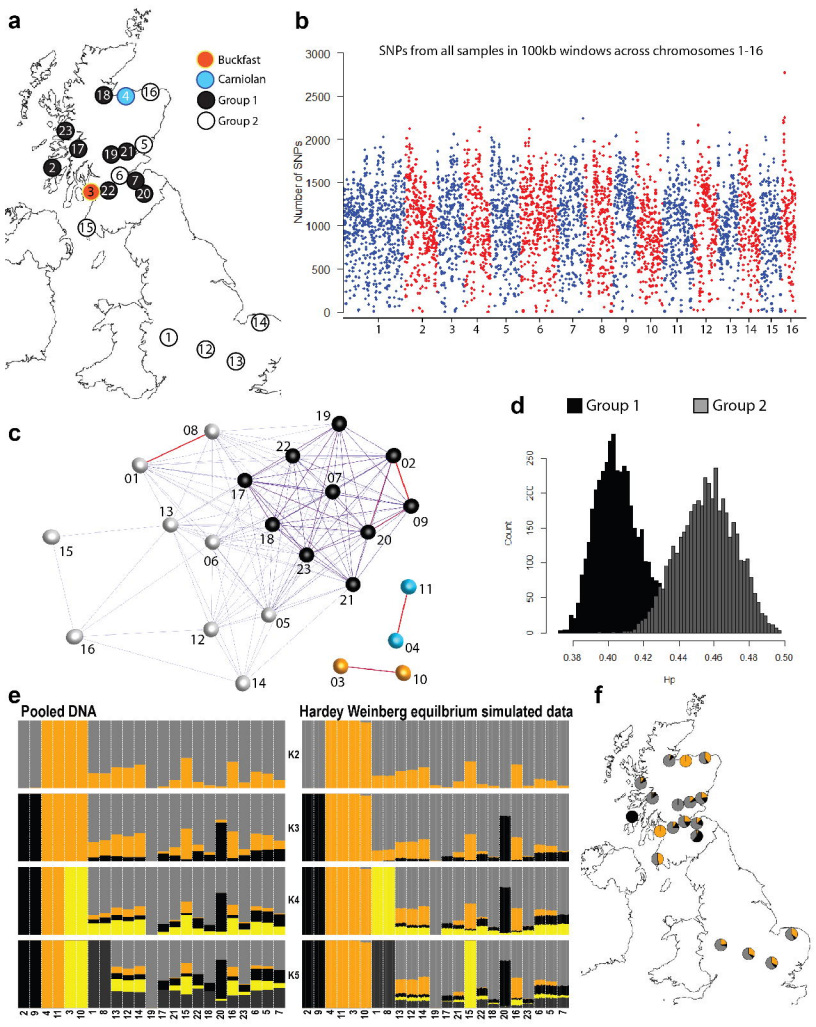
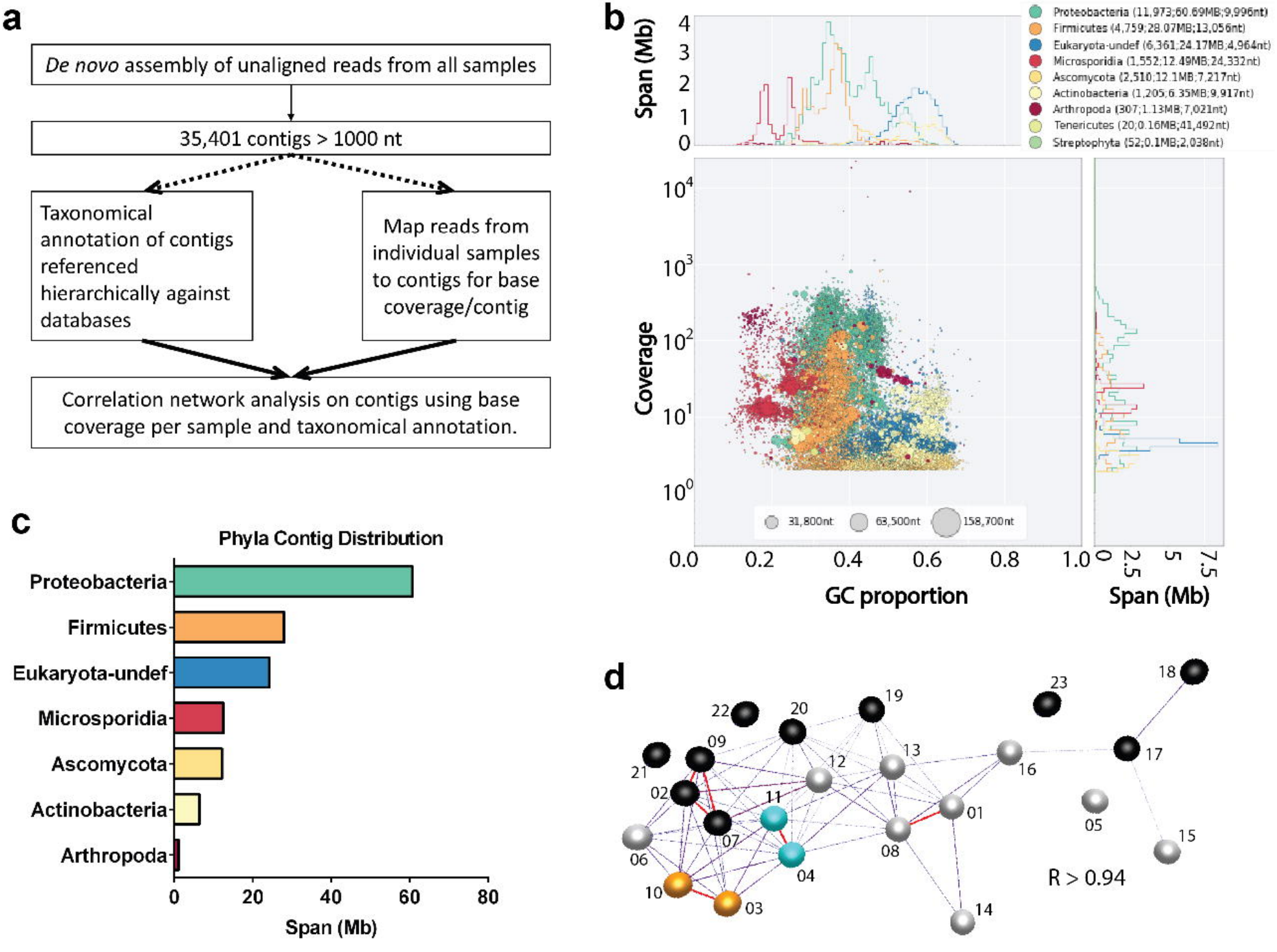
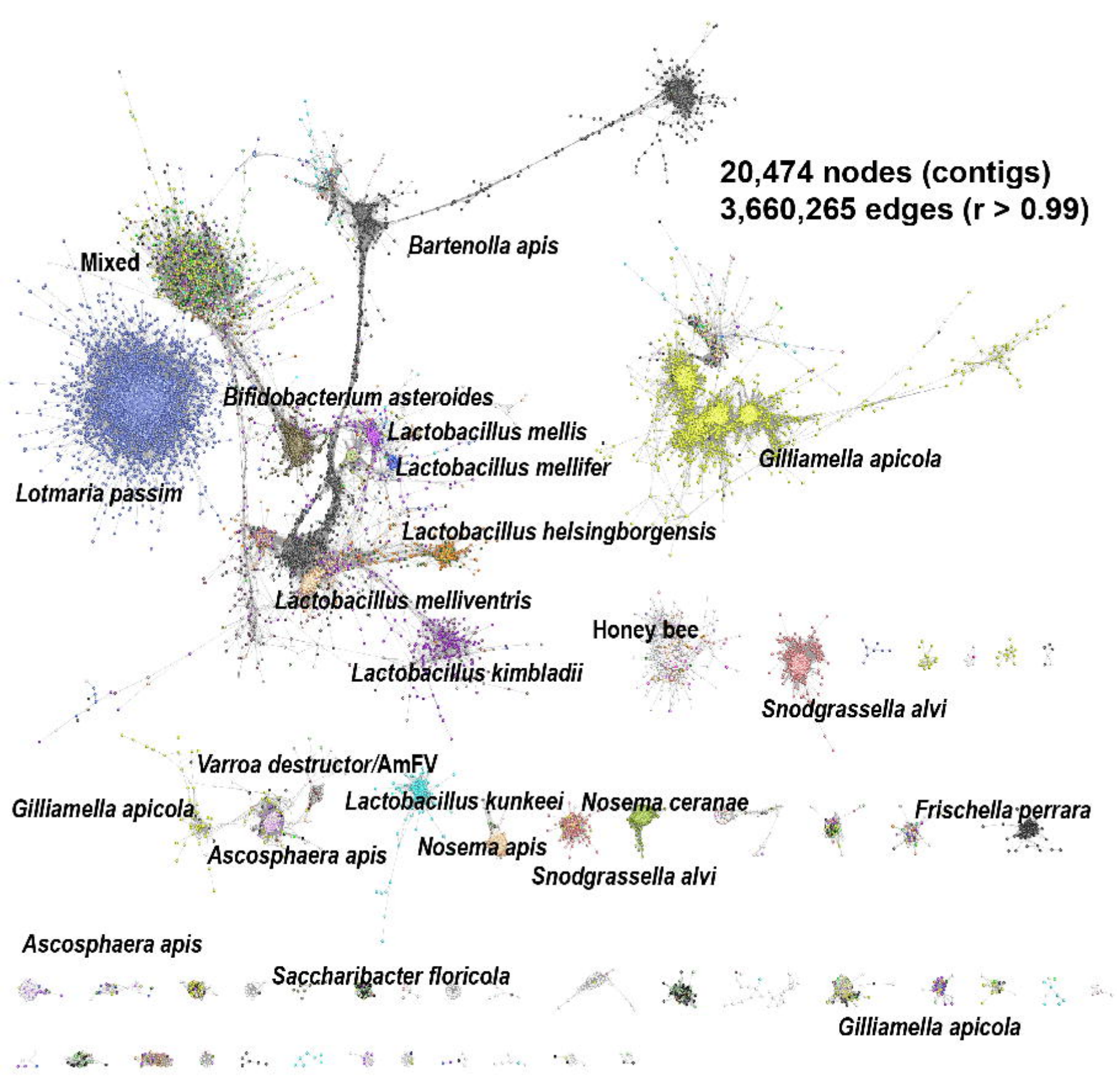
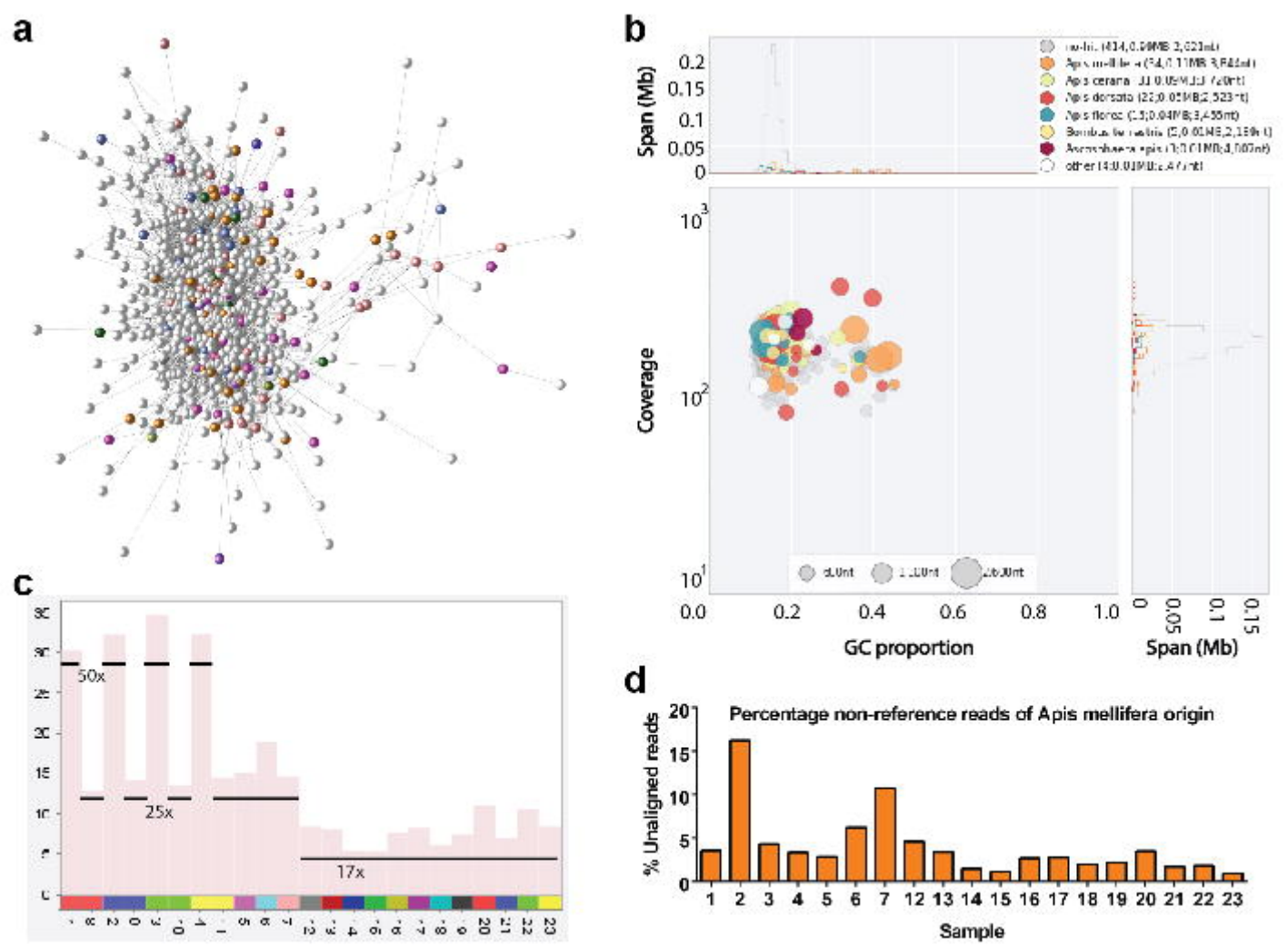
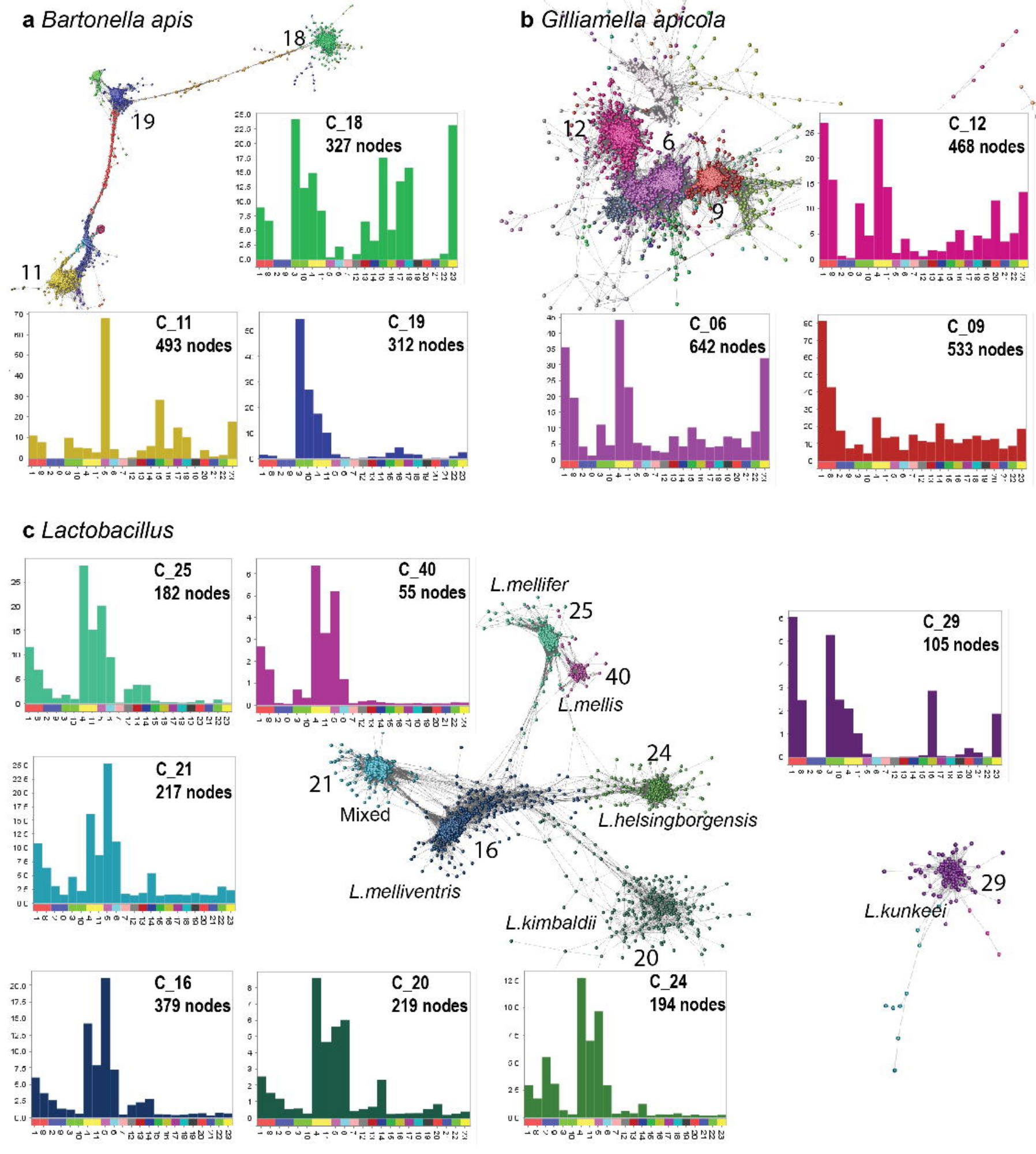
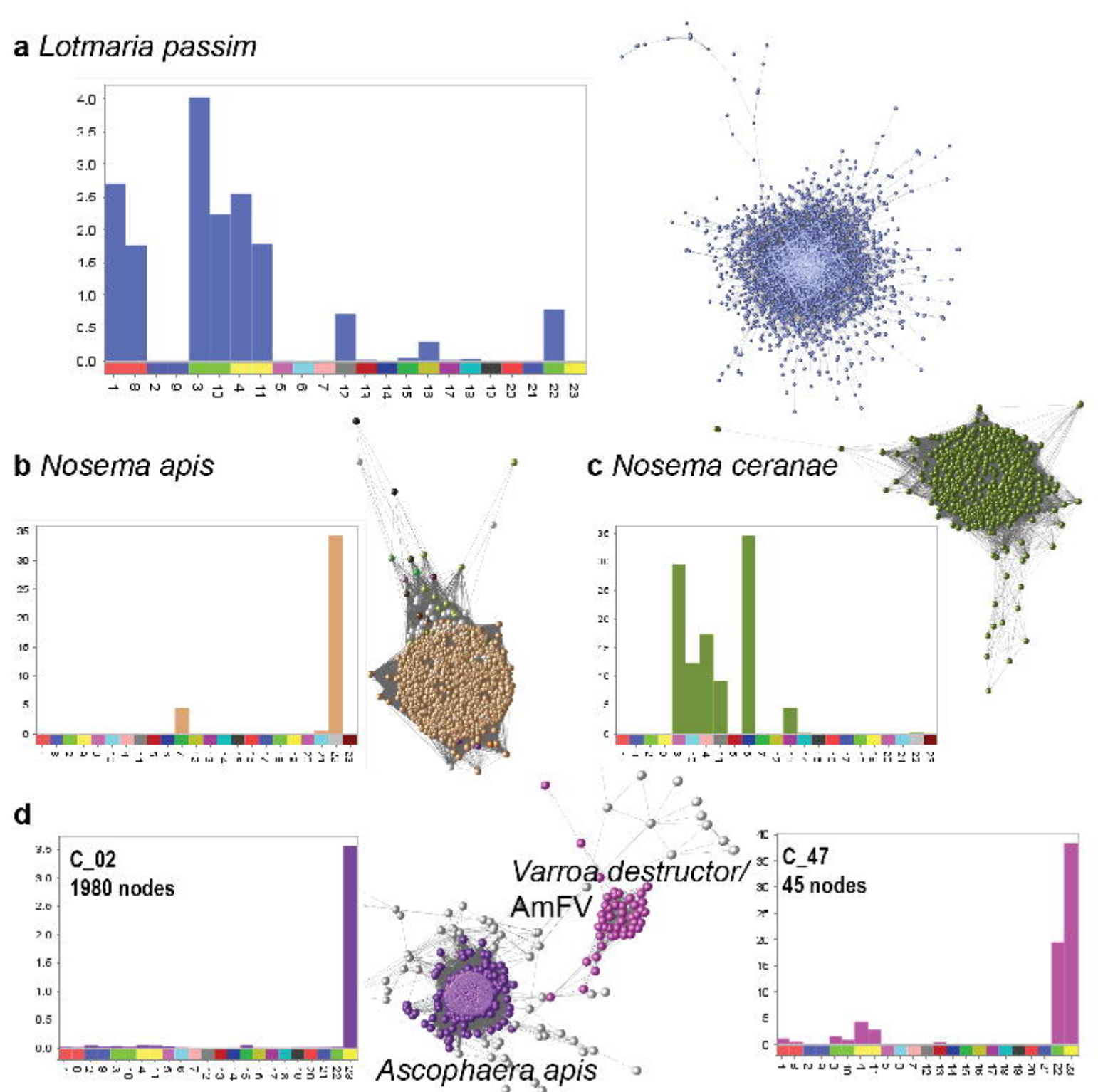

a
Buckfast
Carniolan
Group 1
Group 2
b
SNPs from all samples in 100kb windows across chromosomes 1-16
Number of SNPs
c
d
Group 1
Group 2
Count
Hp
e
Pooled DNA
Hardey Weinberg equilbrium simulated data
K2
K3
K4
K5
f


a
De novo assembly of unaligned reads from all samples
35,401 contigs > 1000 nt
Taxonomical annotation of contigs referenced hierarchically against databases
Map reads from individual samples to contigs for base coverage/contig
Correlation network analysis on contigs using base coverage per sample and taxonomical annotation.
b
Span (Mb)
Proteobacteria (11,973;60.69MB;9,996nt)
Firmicutes (4,759;28.07MB;13,056nt)
Eukaryota-undef (6,361;24.17MB;4,964nt)
Microsporidia (1,552;12.49MB;24,332nt)
Ascomycota (2,510;12.1MB;7,217nt)
Actinobacteria (1,205;6.35MB;9,917nt)
Arthropoda (307;1.13MB;7,021nt)
Tenericutes (20;0.16MB;41,492nt)
Streptophyta (52;0.1MB;2,038nt)
Coverage
31,800nt
68,500nt
158,700nt
GC proportion
Span (Mb)
c
Phyla Contig Distribution
Proteobacteria
Firmicutes
Eukaryota-undef
Microsporidia
Ascomycota
Actinobacteria
Arthropoda
Span (Mb)
d
R > 0.94


20,474 nodes (contigs)
3,660,265 edges (r > 0.99)
Mixed
Bartenolla apis
Bifidobacterium asteroides
Lactobacillus mellis
Lactobacillus mellifer
Lotmaria passim
Gilliamella apicola
Lactobacillus helsingborgensis
Lactobacillus melliventris
Honey bee
Lactobacillus kimbladii
Snodgrassella alvi
Varroa destructor/AmFV
Gilliamella apicola
Lactobacillus kunkeei
Nosema ceranae
Frischella perrara
Ascosphaera apis
Nosema apis
Snodgrassella alvi
Ascosphaera apis
Saccharibacter floricola
Gilliamella apicola


a
b
Span (Mb)
Coverage
GC proportion
Span (Mb)
c
50x
25x
17x
d
Percentage non-reference reads of Apis mellifera origin
% Unaligned reads
Sample


a Bartonella apis
18
19
11
C_18
327 nodes
C_11
493 nodes
C_19
312 nodes
b Gilliamella apicola
12
6
9
C_12
468 nodes
C_06
642 nodes
C_09
533 nodes
c Lactobacillus
C_25
182 nodes
C_40
55 nodes
L.mellifer
25
40
L.mellis
C_29
105 nodes
C_21
217 nodes
21
Mixed
24
L.helsingborgensis
16
L.melliventris
L.kimbaldii
20
29
L.kunkeei
C_16
379 nodes
C_20
219 nodes
C_24
194 nodes


a Lotmaria passim
b Nosema apis
c Nosema ceranae
d
C_02
1980 nodes
C_47
45 nodes
Varroa destructor/
AmFV
Ascophaera apis