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# Genome-resolved metagenomics reveals unexpected diversity and host range of *Candidatus* Lariskella (Rickettsiales: Midichloriaceae)

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**Abstract**

The intracellular endosymbiont *Candidatus* Lariskella (Alphaproteobacteria, *Candidatus* Midichloriaceae) has been found across a wide diversity of terrestrial arthropods, including ticks, true bugs, beetles, fleas, wasps and moths. Despite its prevalence, little is known about the biology of *Ca.* Lariskella, nor do we grasp the full extent of its host range. Here, we report the first known occurrence of *Ca.* Lariskella infecting a population of free-living marine nematodes (Enoplida, Thoracostomopsideae). This novel nematode-infecting *Ca.* Lariskella was found to be closely related to insect-infecting strains, despite the drastic shift in both host taxonomy and habitat. TEM and FISH microscopy showed *Ca.* Lariskella is localized within both the nematode somatic cells and developing oocytes, confirming its status as a nematode endosymbiont and strongly suggesting maternal transmission. This finding led us to reassess the host range of *Ca.* Lariskella. We screened the SRA database for *Ca.* Lariskella sequences and performed genome-resolved metagenomics on SRA entries positive for *Ca.* Lariskella. We recovered 16 novel *Ca.* Lariskella metagenome-assembled genomes from SRA entries, including from novel hosts such as ants and treehoppers. However, we did not encounter further instances of *Ca.* Lariskella within nematodes or marine invertebrates, which we attribute to the relatively poor sampling of these groups. Overall, our findings illustrate the ability of *Ca.* Lariskella to infect both arthropods and nematodes as well as hosts from both terrestrial and marine environments.

**Keywords** *Candidatus* Lariskella, Rickettsiales, Nematode, Host range

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## Introduction

The Rickettsiales are a large, well-studied order of endosymbiotic bacteria within the Alphaproteobacteria. Due to their endosymbiotic nature, Rickettsiales typically possess small genomes (1-1.5 Mb) with streamlined gene contents [1]. Rickettsiales have been widely studied for decades, initially primarily for their role in diseases such as typhus (*Rickettsia prowazekii*), Rocky Mountain spotted fever (*Rickettsia rickettsii*), and anaplasmosis (*Anaplasma phagocytophilum*) [2]. However, diverse Rickettsiales have also garnered increasing evolutionary interest, as their obligate symbiotic lifestyle has resulted in a wide array of unique adaptations, such as supplementing host nutrition [3], aiding host defense [4], manipulating host apoptosis [5], or manipulating host sex determination [6].

*Candidatus* Midichloriaceae (hereafter Midichloriaceae) comprises a recently discovered family within the Rickettsiales order, which was formally described in 2013 [7]. Currently, the Midichloriaceae represent, by far, the least studied among the three families of Rickettsiales, in part due to its novelty, and in part because many Midichloriaceae infect poorly sampled host taxa such as protists [8]. Despite being less studied, Midichloriaceae have been discovered across a vast phylogenetic range of hosts such as in multiple protist supergroups [9–12], placozoans [13, 14], corals [15], multiple arthropods [16–21], as well as in secondary infections of mammals [22–24]. Additionally, Midichloriaceae have been found to exhibit several unique evolutionary traits, such as being the only group in Rickettsiales known to maintain flagellar genes [8, 25, 26], and including the only known bacterial species that can reside within the host mitochondria [21].

Midichloriaceae appear to primarily infect aquatic hosts. However, the family contains two 'terrestrial' genera: *Candidatus* Lariskella (hereafter *Lariskella*) and *Candidatus* Midichloria (hereafter *Midichloria*). Both *Lariskella* and *Midichloria* have primarily been studied in ticks, where they appear to be widespread [17, 21, 27, 28] and are hypothesized to play a nutritional role [29]. Notably, both *Lariskella* and *Midichloria* are transmitted to mammals during the tick's blood meal, though it has not been confirmed whether either bacterium can replicate within mammals or cause disease [22–24].

While *Midichloria* is only known to infect ticks, *Lariskella* is known to infect a far broader range of arthropod hosts. It has been reported in ticks [17, 27, 28, 30], stinkbugs [20], leaf-footed bugs [31], weevils [18], psyllids [19], beetles [16], and fleas [32]. In addition to these published reports, circularized *Lariskella* genomes have been sequenced from wasps (NCBI Refseq GCF_964020185.1) and moths (NCBI Refseq GCF_964019805.1).

Here, we expand the *Lariskella* host range, describing *Lariskella* as an endosymbiont of a marine nematode (Enoplida, Thoracostomopsideae) using a combination of genomics and microscopy. In addition, we screened the SRA database for *Lariskella*, identifying several further novel hosts, including ants and treehoppers, and reconstructed metagenome assembled genomes (MAGs) for multiple *Lariskella* strains. The screening of the SRA did not reveal any further nematode or marine hosts of *Lariskella*, which we hypothesize is due to the limited sampling for these groups. The phylogeny of the novel *Lariskella* MAGs also demonstrated that the nematode-infecting *Lariskella* is closely related to insect-infecting lineages, despite the shift in host and habitat. We hypothesize that this pattern may indicate a relatively recent acquisition by the nematode, supporting the ability of *Lariskella* for host switches.

## Materials and methods

### Nematode sampling

From March 2023 until June 2024, sediment samples were taken from the Okinawan coast (26.4723631548844, 127.83168712213792). Nematodes were extracted from the sediment through a magnesium chloride decanter method: 100 mL of 7.5% MgCl2 was added to approximately 200 mL of sediment. Sediment was stirred with a spoon and left to sit for 10 min, after which it was stirred again, and the solution was sieved through a 32 µm sieve. The contents of the sieve were washed into a Petri dish using sand-filtered seawater. Nematodes were then picked from the Petri dish using an Irwin loop under a dissecting microscope (Olympus SZX16). Nematodes picked for microscopy were washed once in filter-sterilized (0.22 µm) seawater to remove debris and then transferred directly to the appropriate fixative. All other nematodes were cleared of surface contaminants by a triple serial transfer in filter-sterilized seawater using sterile pipette tips. Cleaned nematodes were then frozen at −70°C awaiting processing.

### Illumina sequencing

For 15 nematodes, shotgun metagenomes were obtained using Illumina sequencing. DNA was extracted from the nematodes using the MasterPure Complete DNA and RNA purification kit (Thermo Fisher Scientific). The kit was used following the manufacturer's instructions, with the exception of a prolonged (2 h) proteinase K incubation to facilitate full lysis of the nematode. Illumina libraries were prepared using the NEBNext Ultra II PCR plus kit (New England Biolabs) and sequenced on the SP flow cell of the NovaSeq 6000 platform with 150 bp paired-end reads. The generated reads were thereafter quality checked using Fastqc v0.11.9 (https:/www.bioinformatics.babraham.ac.uk/projects/fastqc/).

### Lariskella identity and diagnostic PCR

The initial identification of *Lariskella* was performed by 16S rRNA gene reconstruction using phyloFlash. To experimentally confirm the presence of *Lariskella*, a diagnostic PCR with *Lariskella*-specific primers was performed in 10 nematodes. We amplified the *Lariskella rrl-rrf* intergenic spacer using the *Lariskella*-specific Mtz-5S-F and Mtz-23S-R primers [33] and Q5 high-fidelity DNA polymerase (New England Biolabs), with the following thermocycling conditions: initial denaturation at 94 °C for 4 min; then 30 cycles of 94 °C for 1 min, 62 °C for 1 min, 72 °C for 80 s; followed by a final elongation at 72 °C for 4 min. To confirm that the bands were the intended products, the obtained bands were Sanger sequenced as described above. Resulting Sanger sequences were compared against the *Lariskella* rRNA operon obtained during Illumina sequencing using EMBOSS Needle [34], to ensure the PCR product originated from the *Lariskella* symbiont.

### Lariskella genome binning

Based on the 16S and 18S rRNA gene sequences, both the host ($N$ = 15) and *Lariskella* ($N$ = 4) were identical across the shotgun-sequenced nematode samples. The reads for these samples were thereafter trimmed using Fastp v0.23.2 [35] and co-assembled using Megahit v1.2.9 [36]. The coverage for this assembly was then calculated by Bowtie2 v2.4.2 [37], and sorted, compressed, and indexed using SAMtools v1.12 [38]. The assembly was then binned using Metabat2 v2.12.1 [39], CONCOCT v1.1.0 [40], and SemiBin2 v2.0.2 [41], and the resulting bins were refined with DASTool v1.1.6 [42]. It was then assessed which of the metagenome assembled genomes (MAGs) corresponded to *Lariskella* through a threefold method: first, a MEGAN v6.23.3 [43] lowest common ancestor assignment analysis was run to confirm the approximate composition of the MAG. Second, the 16S rRNA genes in the MAGs were predicted using barrnap v0.9 (https://github.com/tseemann/barrnap), and aligned by BLASTn v2.10.0 [44] against the 16S rRNA gene reconstructed by phyloFlash. Finally, the MAG was aligned with the Mummer nucmer algorithm v3.23 [45] against the complete *Lariskella* reference genomes obtained from NCBI (GCF964019805.1 and GCF964020185.1). For all MAGs of *Lariskella* origin, the best MAG was chosen based on completeness and duplication scores inferred by CheckM2 v1.0.2 [46]. The best quality MAG was found to originate from CONCOCT. CONCOCT filters out contigs smaller than 1000 bp by default, and these are thus not present within the *Lariskella* MAG.

### SRA screening and Lariskella phylogeny

The NCBI SRA database was screened for *Lariskella* by querying the STAT assignments of SRA entries for *Lariskella* using bigQUERY on Google Cloud. Entries that had at least 1000 reads assigned as *Lariskella* were downloaded and checked for *Lariskella* 16S rRNA gene sequences using PhyloFlash. Entries for which a *Lariskella* 16S rRNA gene was recovered were binned using the method described above. The MAGs corresponding to *Lariskella* were then identified using GTDB-Tk v2.4.0 [47], and their quality determined using CheckM2.

The phylogenetic positions of the nematode MAG, the SRA *Lariskella* MAGs, and the two publicly available *Lariskella* genomes (GCF_964020185.1 and GCF_964019805.1) were inferred from a multigene phylogenetic tree. Prokka v1.14.6 [48] was used to predict protein-coding sequences, which were piped into Orthofinder v2.5.4 [49] to find single-copy orthologs shared across the 25 genomes. The protein sequences of the 114 shared single-copy orthologs were aligned using MAFFT v7.505 [50], set to -localpair and with 1,000 iterations. The alignment was then trimmed using trimAl v1.4.rev22 [51] set to automated1, and concatenated using a Python script. A maximum likelihood phylogenetic tree was reconstructed from the concatenated alignment using IQ-TREE2 v2.0.7 [52], with 1,000 bootstraps. IQ-TREE2 model finder chose JTTDCMut+F+R3 as the best model based on the Bayesian Information Criterion.

### Host 18S rRNA gene sequencing and phylogeny

The 18S rRNA gene sequences of the nematode host were assembled from the metagenomes and approximately identified using phyloFlash v3.4 [53]. To determine the host's exact phylogenetic position, an 18S rRNA gene tree was reconstructed. 18S rRNA gene sequences of nematodes from Enoplida were downloaded from SILVA [54], with Triplonchida 18S rRNA gene sequences used as an outgroup. The downloaded sequences were aligned with the metagenome-reconstructed 18S rRNA gene sequence using MAFFT set to a maximum of 1,000 iterations, and using the -localpair argument. A phylogenetic tree was reconstructed from the alignment using IQ-TREE2 set to 1,000 bootstraps using the GTR + F + R4 model selected by the IQ-TREE2 modelfinder based on the Bayesian Information Criterion. As a comparison, trees were also reconstructed using the GTR + G + I model frequently used in nematode 18S rRNA gene phylogeny literature [55, 56] and both trees had identical topologies with only minor differences in branch lengths (Supplemental figure S1). We confirmed the similarity of the 18S rRNA sequences using BLASTn megablast on the NCBI website (3-6-2025) [44].

### Double labelling of oligonucleotide probe for fluorescence in situ hybridization (DOPE-FISH) microscopy

Multiple nematodes (5–20 individuals per slide) were picked and placed in a 1 mL drop of filter-sterilized (0.22 µm) sea water. They were then transferred to a 1.5 mL tube containing 100 µl of 1:1 glacial acetic acid: ethanol solution, where they were fixed for 10 min. Fixed nematodes were washed in 100% ethanol two times for 5 min, and incubated in 1:1 methanol: PBS-T (1X PBS buffer containing 0.1% Tween 20) for 10 min. Afterwards, they were incubated in 1% paraformaldehyde (Nacalai) PBS-T, which was washed away with two PBS-T washes of 2 min each. Nematodes were prepared for hybridization with three washes in hybridization buffer (0.9 M NaCl, 0.02 M Tris-HCl, 0.01% sodium dodecyl sulphate (SDS, Sigma-Aldrich) + 30% formamide; Wako) of 5 min each. The nematodes were then hybridized in 100 µL of hybridization buffer containing DAPI DNA stain (0.01 µg/µL; Roche), and 0.1 µM of *Lariskella*-specific probe (JBioS) overnight at 42°C. The Lariskella-specific probe (CTGT GATAGTCCGGCCGAAC) was purified through high performance liquid chromatography purification. This probe was then labelled at both the 5' and 3' ends with Alexa Fluor 647. The DNA sequence of the probe was determined to be fully *Lariskella*-specific using SILVA's TestProbe v3.0 function [57]. After hybridization, excess DAPI and probe were washed away with three PBS-T washes of 10 min. Nematodes were suspended in MilliQ water and pipetted onto a glass slide. After removing excess water, the nematodes were mounted using ProLong Diamond Antifade Mountant (Thermo Fisher Scientific) and imaged using a confocal fluorescence microscope (Microscope: Nikon A1, detector: x2 MA PMT, x2 GaAsp, x1 T-PMT, lasers: 404.6 nm and 637.8 nm).

### Transmission electron microscopy (TEM)

Five adult female nematodes were picked from sediment samples. These nematodes were fixed by briefly (2 min) submerging in the fixative (2.5% glutaraldehyde; Fujifilm Wako and 2% paraformaldehyde; Fujifilm Wako in 0.1 M HEPES; Nacalai). The anterior of the nematode (from the mouth to slightly behind the end of the pharynx) was dissected from each nematode. The posterior of the nematodes was incubated in the fixing solution overnight at 4 °C, awaiting TEM post-fixation steps. The processed nematodes were assessed for *Lariskella* infection by performing the *Lariskella* diagnostic PCR described above on the dissected pharynges. One nematode was found to be *Lariskella*-positive, and its posterior was prepared for TEM.

The posterior of the *Lariskella*-positive nematode was washed with 0.1 M HEPES for 5 min three times, and post-fixed with 2% osmium tetroxide for 1 h at room temperature. After post-fixation, the nematode was *en bloc* stained with 2% uranyl acetate overnight at 4 °C. The nematode was dehydrated by serial transfer through increasing concentrations of ethanol (30%, 50%, 70%, 90%, 100%), incubating for 10 min in each, and two times 10 min in 100% ethanol. This was followed by a final dehydration using 100% acetone for two times 10 min. Low viscosity resin (Agar Scientific, catalog no. R1078) was then infiltrated by replacing with 2:1 (vol: vol) of acetone: resin for 2 h at room temperature while rotating in an TAITEC Rotator RT-50. After 100% of the resin was replaced, the resin was infiltrated under a 1-hour vacuum and incubated overnight at room temperature. The resin was then polymerized for 48 h at 60°C. The resin block was trimmed using glass knives, and ultrathin sections (50–60 nm) were cut using a Leica Ultra-microtome EM (UC7). Ultrathin sections were collected onto the 0.5% formvar (SPI-CHEM) coated copper one-slot grids (Nisshin-EM). The air-dried grids were then examined using a JEOL 1400 Flash transmission electron microscope.

### Sex-specific infection rate experiment

*Lariskella* diagnostic PCR was performed on adult nematodes of known sex to determine per-sex infection rates. Nematodes were collected, and their sex was determined based on morphology using an inverted microscope (Nikon Eclipse Ti2-E). Due to the difficulty of determining sex in juveniles, only adults were considered. Adults with a sclerotized gubernaculum were determined as males. The gubernacula had clear autofluorescence under 488 nm light, which was used to more easily identify adult males. Adults lacking a gubernaculum, but containing ovaries with visible eggs, were considered as fully developed adult females. No hermaphrodites were observed. The presence of *Lariskella* was then confirmed in the sexed nematodes using the *Lariskella* diagnostic PCR described above. In total, 35 sexed nematodes were tested with PCR (23 females, 12 males). Furthermore, where possible, adult nematodes from FISH slides were sexed as well, and assessed visually for *Lariskella* signal. In total, 23 sexed nematodes were assessed using FISH (16 females, 7 males). To assess whether male and female infection status followed the same distribution, chi-square goodness of fit tests were performed on the PCR and microscopy results individually.

### Analysis of reproductive manipulation gene presence in Lariskella

To identify potential reproductive manipulation genes in the nematode-infecting *Lariskella*, we compiled a curated reference gene set (Supplemental sequences) including putative male-killing genes (*wmk*, *oscar*, *Spaid*), cytoplasmic incompatibility genes (*cifA* and *cifB*), and parthogenesis induction genes (*pifB* and *piff*). We generated custom

BLAST databases containing these reference genes. Protein-coding genes from *Lariskella* were predicted using Prokka and queried against this database using BLASTp (E-value ≤ $1e^{-5}$, minimum sequence identity ≥ 30%). Only *wmk*-like genes resulted in BLAST hits above 50% identity.

The protein domains present in the inferred *wmk*-like orthologs were identified using InterProScan v5.60-92.0 [58]. InterProScan reported only 1 domain shared among all *wmk* orthologs, which was annotated as a Cro/C1-type DNA-binding domain. Alphafold2 v2.1.1 [59] was used to infer the protein structures, which were visualized in iCn3D [60]. Structural alignments were performed using TM-align. These structural alignments showed a helix-turn-helix structure shared among all identified orthologs, the position of which corresponded to the Cro/C1-type DNA-binding domains reported by InterProScan.

#### Functional annotation of Lariskella genomes

The *Lariskella* MAGs and the two reference *Lariskella* genomes were annotated using eggNOG-mapper v2.1.12 [61] in genome mode, using the bundled MMseqs2 BLASTx as the gene prediction method, and the eggNOG bacterial database. The eggNOG-mapper COG assignments were extracted and counted using a Python script.

ANI was assessed using ANIclustermap (https://github.com/moshi4/ANIclustermap) using the default settings, including ANI calculation by fastANI [62].

To infer the potential nutritional contribution by *Lariskella*, the completion of metabolic pathways synthesizing nutrients essential for nematodes was assessed using the genome data. Nematode essential nutrients were based on *Caenorhabditis elegans* as a guide [63]. The KEGG KO numbers were extracted from the eggNOG-mapper annotation using a Python script, and used to assess the completion of the KEGG modules corresponding to the synthesis of essential nutrients in KEGG mapper reconstruct [64, 65]. If there were multiple redundant KEGG modules, only the most complete bacterial pathway was considered.

## Results

#### Lariskella identity, genome and phylogeny

The *Lariskella* infection within this nematode population was first discovered by the presence of a *Lariskella arthropodarum*-related (99.6% identity) 16S rRNA gene in the nematode metagenome. Diagnostic PCRs with *Lariskella*-specific intergenic primers were performed to experimentally confirm the presence of *Lariskella*. In total (including the sex-specific infection rate experiment), diagnostic PCRs were performed on 50 individuals, of which ten gave visible bands indicating *Lariskella* infection.

Using binning, a MAG of the suspected *Lariskella* was obtained from 15 co-assembled nematode metagenomes. This MAG was compared against two publicly available *Lariskella* genomes. The MAG is fragmented (321 contigs) but is highly similar to the references in assembly size (1.5 Mb), CheckM2 completeness score (96.9%), and GC content (35%) (Supplemental Table S1). The MAG was aligned against the references and showed a high degree of nucleotide-level similarity, further confirming its identity as *Lariskella* (Supplemental Figure S2).

To assess the phylogenetic position of this novel *Lariskella* lineage, we reconstructed a 114-gene multigene phylogenetic tree using 2 NCBI *Lariskella* references and 16 MAGs reconstructed from SRA data (Fig. 1A). In this tree, the nematode *Lariskella* is nested within insect-infecting lineages, clustering most closely to a *Lariskella* reference genome infecting Lepidoptera.

We also reconstructed a phylogenetic tree based on the 18S rRNA gene of the nematode host (Fig. 1B). While the host 18S rRNA gene did not match exactly with any known reference, the phylogenetic tree places it within the family Thoracostomopsidae of the order Enoplida. The nematode clusters closest to a *Mesacanthoides* species with 97% sequence identity.

#### DOPE-FISH microscopy and TEM

The localization of the novel *Lariskella* strain was visualized using DOPE-FISH microscopy (Fig. 2A-C). The localization of the *Lariskella*-specific probe shows *Lariskella* has a patchy distribution of intracellular infections throughout the somatic tissue of the nematode host. The ovaries of the nematode clearly deviate from this patchy distribution, and instead display highly abundant infections of *Lariskella* in both the maternal ovarian tissue and the developing oocytes.

To further confirm the intracellular nature of the nematode *Lariskella*, TEM micrographs of various nematode tissues were inspected. The micrographs show the presence of bacteria-like structures within the oocytes (Fig. 2D-E), as well as within the somatic cells (Supplemental Figure S3).

#### Sex-specific infection rates

Diagnostic PCR showed an infection rate of 26% for female nematodes (6/23 infected) (Fig. 3). Adult males were found to be free of infection, with none of the tested males ($N = 12$) showing bands during the PCR. These results matched the DOPE-FISH results, where no *Lariskella* signal was observed in any of the processed males ($N = 7$). In contrast, 7 out of 16 adult females showed a clear *Lariskella* signal.

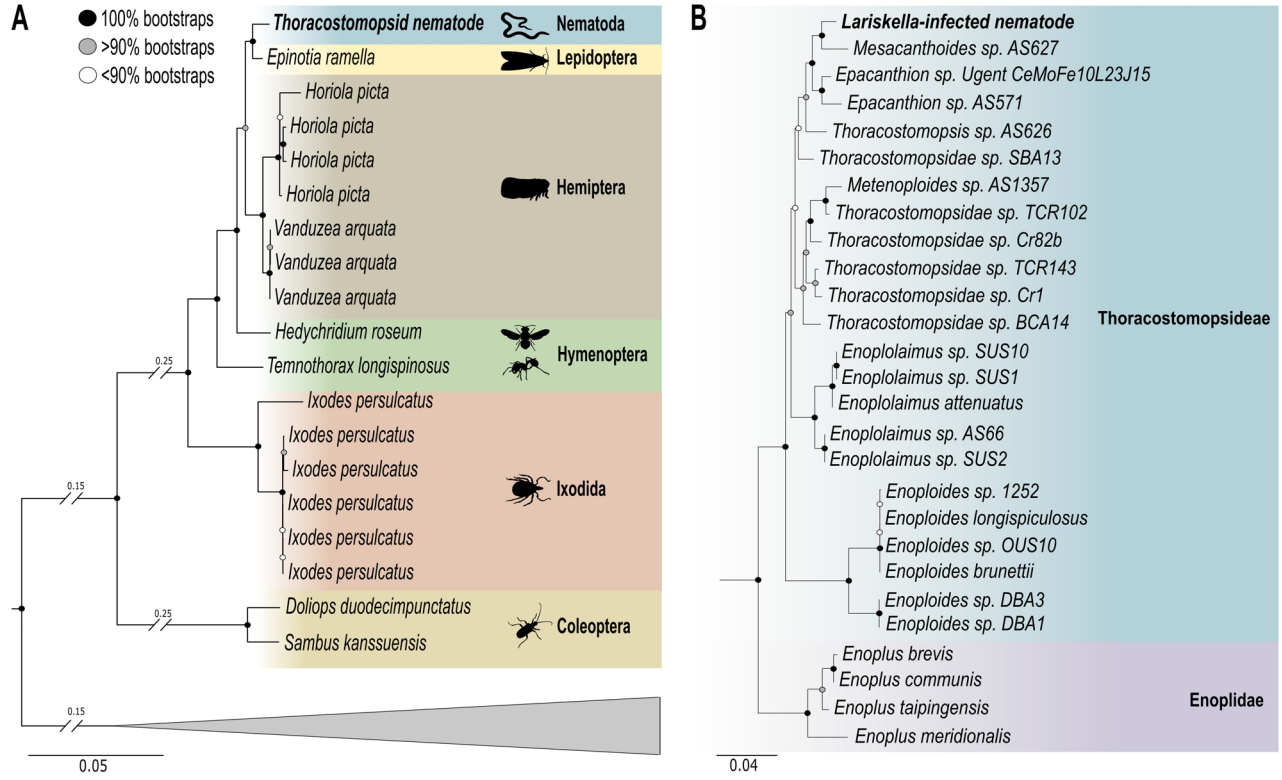


**Fig. 1** **A**) 114-gene multigene phylogenetic tree of the genus *Lariskella*, showing the position of the assembled *Lariskella* MAGs, named based on their host species. The tree was inferred by maximum-likelihood based on the JTTDCMut + F+R3 model, and rooted with *Cytobacter* and *Midichloria* species as outgroups. **B**) Cropped phylogenetic tree based on nematode 18 S rRNA gene sequences, demonstrating that the nematode host belongs to the Thoracostomposidae. The tree was inferred by maximum-likelihood based on the GTR + F+R4 model. The full tree is shown in Supplemental Figure S1

### SRA screening

To determine the prevalence of *Lariskella* infection in nematode or marine hosts, we screened the complete NCBI SRA database for *Lariskella* sequences (Supplemental Table S2). We found 305 entries that contained at least 1000 reads that were identified as potential *Lariskella*. For 33 of these entries, a *Lariskella* 16S rRNA gene was recovered from the metagenome. From 16 entries, high-quality (>80% completion) *Lariskella* MAGs were recovered (MAG statistics in Supplemental Table S1). We assessed the COG counts, completion, and genome sizes of these MAGs (Fig. 4). None of the SRA entries that yielded *Lariskella* MAGs or 16S rRNA gene sequences were of aquatic or nematode origin. Instead, the *Lariskella* MAGs originated from a variety of terrestrial arthropods: treehoppers (*Horiola picta*, *Vanduzea arquata*), ants (*Temnothorax longispinosus*), ticks (*Ixodes persulcatus*), and beetles (*Doliops duodecimpunctatus*, *Sambus kanssuensis*). Of these, treehoppers and ants were not previously known to harbor *Lariskella.*

Notably, the diversity of *Lariskella* MAGs appears to constitute several distinct species of the *Lariskella* genus. According to an ANI analysis (Supplemental Figure S4), the *Lariskella* strains within ticks comprise a distinct species from the other hosts. Arguably, the *Lariskella* within *Temnothorax longispinosus* might also constitute its own species. *Lariskella* from treehoppers, moths and the marine nematode appear to form a single species. The MAGs obtained from beetles were identified as *Lariskella* by GTDB-Tk, but have less than 80% identity with any other *Lariskella* genome. Thus, these beetle-derived lineages appear to instead comprise a sister genus, phylogenetically close but distinct from *Lariskella*.

### Lariskella metabolic capacity

The *Lariskella* genomes encode complete pathways required to synthesize the essential vitamins pyridoxine (B6) and biotin (B7) (Fig. 5). However, none of the pathways for essential amino acids were fully encoded by any of the *Lariskella* genomes. The pathways for lysine, heme, and tetrahydrofolate are nearly complete, and could potentially be completed by uncharacterized enzymes or through substrate exchange with the host. The nematode-infecting *Lariskella* appears not to deviate from the metabolic profiles of other *Lariskella.* The *Lariskella*-allied lineages infecting beetles do slightly deviate, as these appear to encode more complete pathways for nicotinamide and arginine than found for the *Lariskella* lineages.

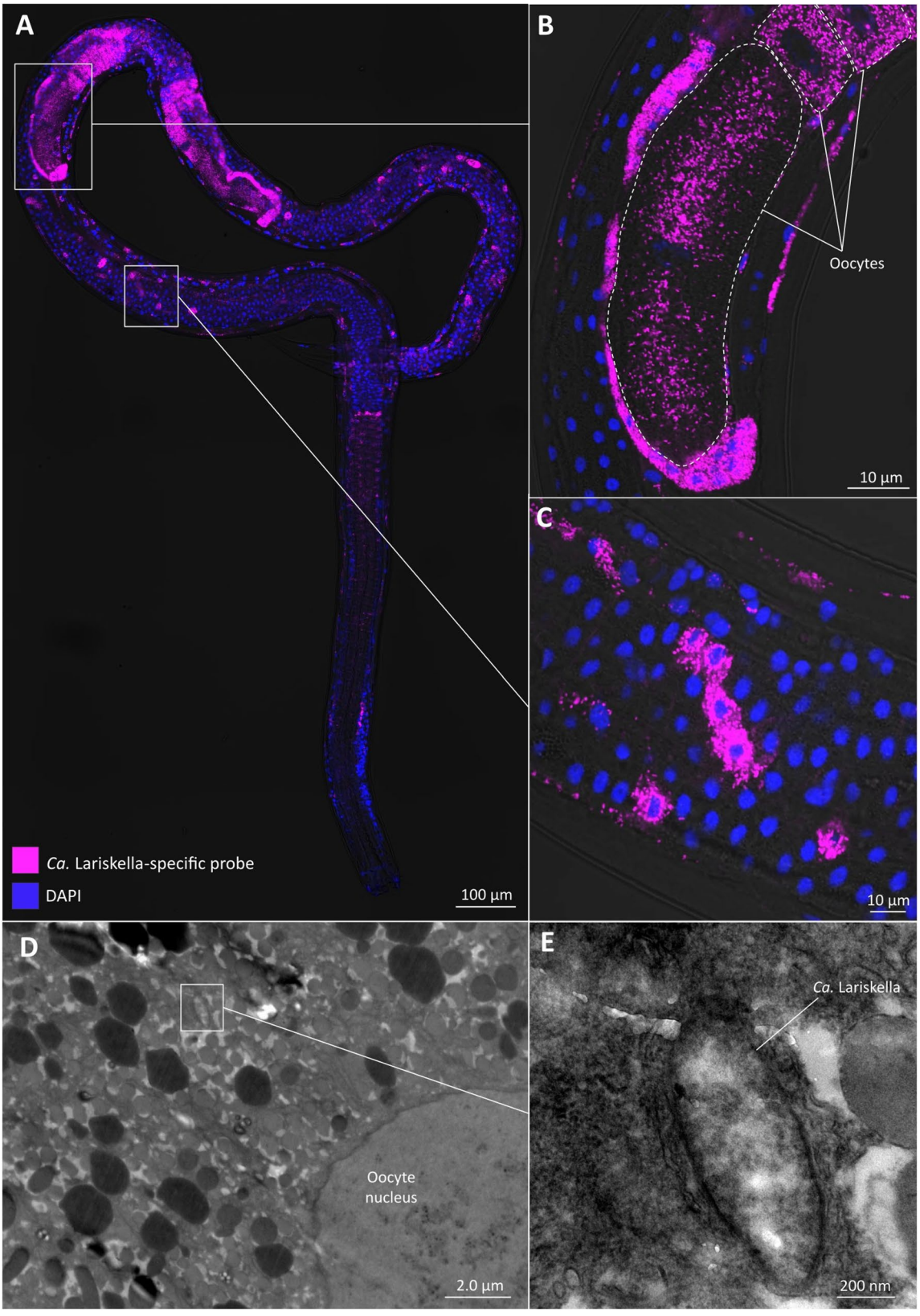


**Fig. 2** **A** to **C**) DOPE-FISH microscopy images of a whole-mounted gravid female nematode. Pink indicates the *Lariskella*-specific probe. Blue indicates DAPI, which stains all DNA and visualizes host nuclei. **A**) Whole nematode. **B**) Zoomed image of one of the paired ovaries, including infected oocytes **C**) Zoomed image of somatic tissue, including gut epithelium and cuticle. **D**) and **E**) TEM micrographs of a nematode oocyte. **D**) Electron micrograph showing the inside of an infected oocyte. **E**) Electron micrograph within an oocyte, zoomed in on a *Lariskella* cell

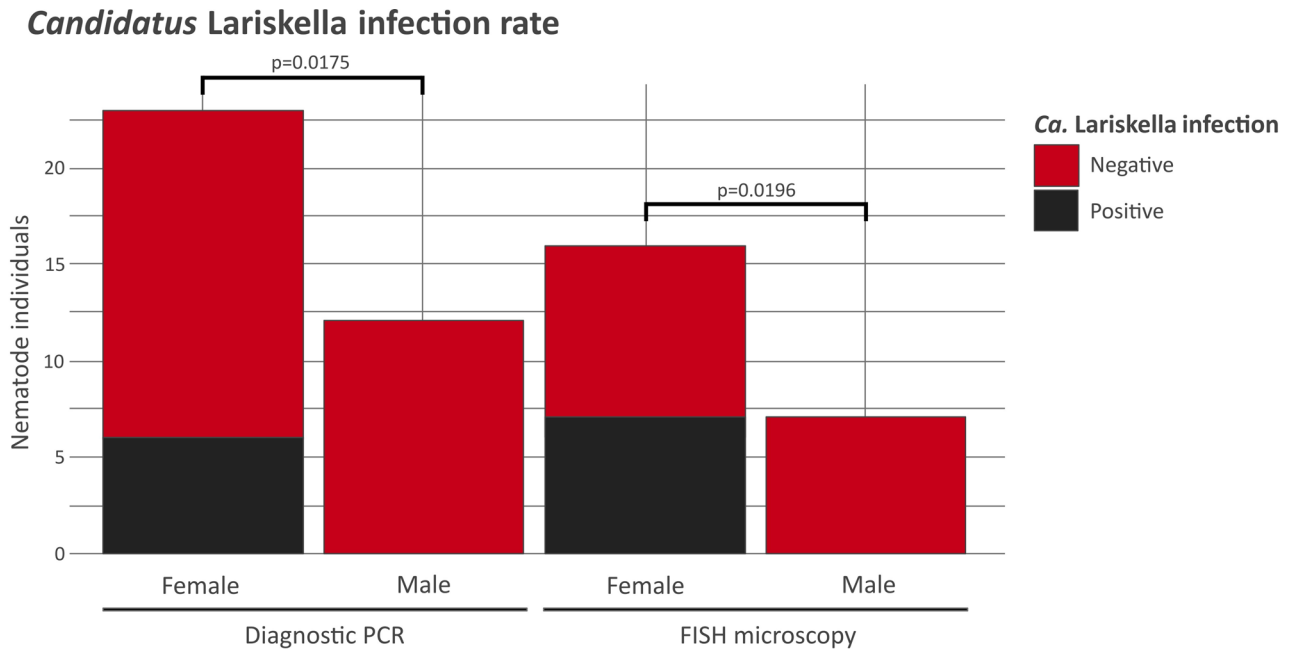


**Fig. 3** Bar plot displaying the infection status of tested nematodes. Sexed nematodes were examined for *Lariskella* infection using diagnostic PCR ($N = 35$), and DOPE-FISH microscopy ($N = 23$). For both the PCR and microscopy experiments a chi-square goodness of fitness test confirmed a significant difference in the female and male infection status (PCR: $p = 0.0175$, microscopy: $p = 0.0196$)

## Discussion

In this study, a wild population of marine nematodes was confirmed to be infected by *Lariskella* through multiple experiments. The presence and identity of the *Lariskella* symbiont were confirmed through metagenomics, diagnostic PCR, and DOPE-FISH microscopy. In addition, the intracellular localization of the nematode-infecting *Lariskella* was confirmed using DOPE-FISH microscopy and TEM. All results point towards the *Lariskella* being a vertically transmitted intracellular symbiont of the nematode host. This novel host represents the first known nematode host of the genus *Lariskella*, as well as the first known nematode host for bacteria of the family *Midichloriaceae*. Additionally, this marine nematode represents the first known aquatic host found for *Lariskella*, which was previously considered exclusively terrestrial [8].

### The phylogeny suggests a recent infection of nematodes

It was unexpected for the nematode-infecting *Lariskella* to be so closely related to the insect-infecting *Lariskella*. Nematodes occur in both marine and terrestrial environments, and throughout their evolution have frequently transitioned between these two habitats [66]. Therefore, we initially suspected that marine nematodes were ancient hosts of *Lariskella*, potentially representing a link from the ancestrally marine hosts of Midichloriaceae to the primarily terrestrial hosts of *Lariskella*. However, the phylogenetic tree contradicts this notion, with the nematode-infecting *Lariskella* instead nested among the insect-infecting *Lariskella* lineages. This nested placement of *Lariskella*, combined with its high nucleotide-level similarity to insect-infecting *Lariskella* strains, suggests the nematode population was likely infected by *Lariskella* relatively recently. Since the Thoracostomopsidae are primarily predators [67], and as predation can facilitate the horizontal transmission of endosymbionts [68], we suspect the nematode population might have been infected through the ingestion of infected arthropods.

### Microscopy confirms an intracellular localization

Microscopy confirmed the identity of the symbiont as *Lariskella*, with clear binding of the *Lariskella*-specific probe. The bacterial morphology in the TEM micrographs also resembled the *Lariskella* observed in insect cells [20], though the nematode-infecting *Lariskella* are notably more compact and less filamentous. Additionally, the microscopy demonstrated the intracellular localization of the *Lariskella* symbionts, confirming *Lariskella* represents a true intracellular infection of the nematodes and did not originate from external contamination. Furthermore, both the TEM and DOPE-FISH microscopy confirmed *Lariskella* colonized the developing oocytes within the gravid females, strongly suggesting maternal transmission of *Lariskella*. Additionally, all oocytes within infected females displayed abundant *Lariskella* signal, suggesting a high rate of transmission. However, despite this suspected transmission efficiency, the overall *Lariskella* infection rate in nematodes was surprisingly

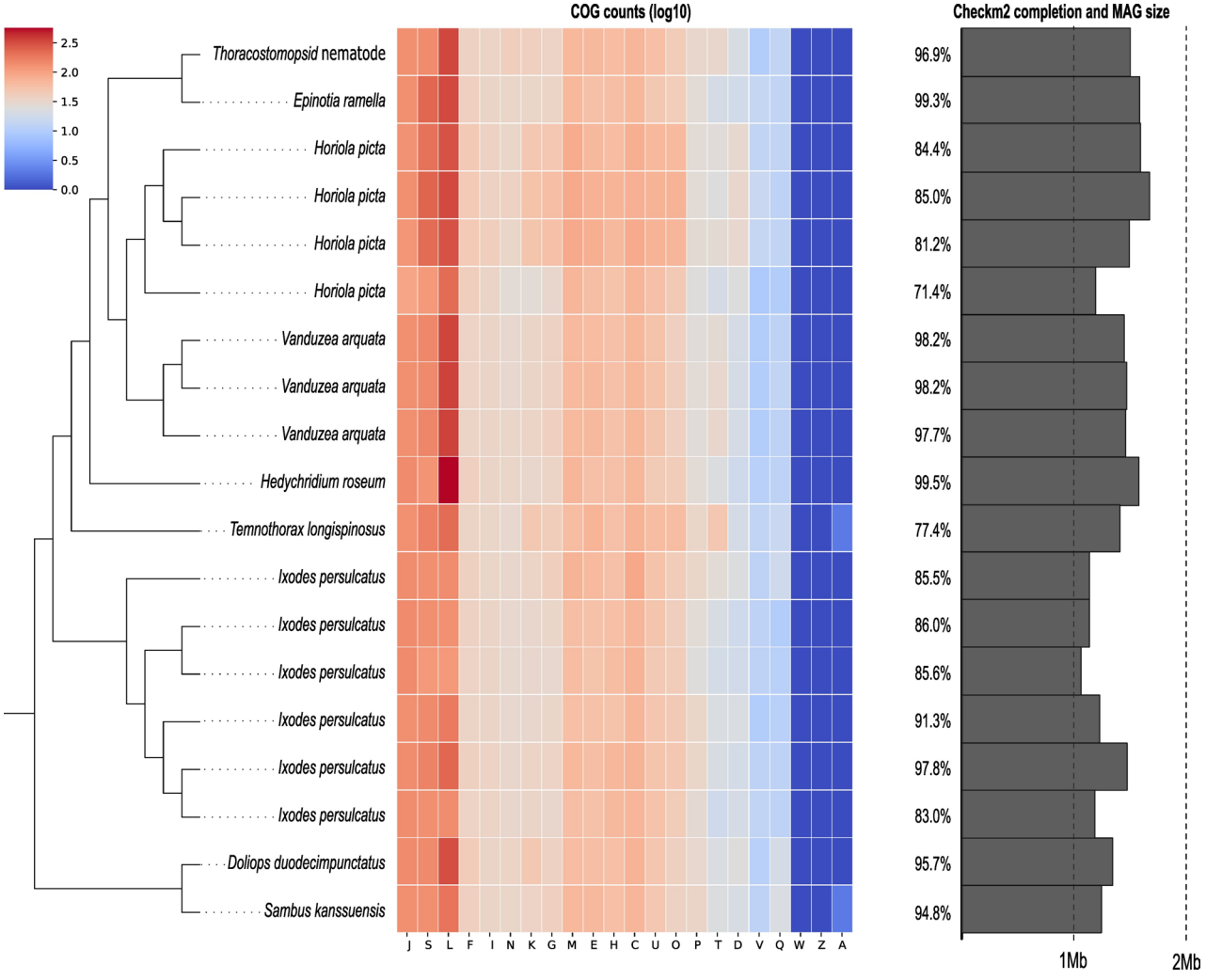


**Fig. 4** Heatmap showing the COG counts (logarithmically scaled) found per *Lariskella* genome or MAG, named according to their host species. The bar plot shows the genome size and Checkm2 completion per assembly. Genomes were clustered based on their phylogeny described in Fig. 1

low. Most notably, male nematodes seemed to be entirely free of infection during DOPE-FISH microscopy (Supplemental Figure S5). While some *Lariskella* probe signal could be observed within males (Supplemental Figure S6), these signals did not form clear cell-shaped structures or co-localized with the DAPI stain, and thus most likely originated from incomplete washing of unbound probe. This was confirmed with diagnostic PCR which also failed to detect *Lariskella* within male nematodes.

### The observed Lariskella infection rate might suggest reproductive manipulation

The overall infection rate of the nematode was found to be approximately 20%. This is far lower than infection rates typically reported for *Lariskella*, with studies reporting *Lariskella* frequencies around 47%-52% in ticks [33, 69, 70], 77%-100% in *Nysius* stinkbugs [20], 66%-100% in leaf-footed bugs [31], and 100% in psyllids [19]. Additionally, our results suggest that all male nematodes were free of *Lariskella* infection. A reduced prevalence of *Lariskella* in males is a known phenomenon. Female ticks have abundant *Lariskella* infections, while males only harbor small to negligible numbers of *Lariskella* [17, 28, 71]. Becker et al. (2023) suggested that *Lariskella* might be maternally transferred to all offspring, but that the *Lariskella* titer diminishes in males over the course of their development [28], similar to what has been observed for the related bacteria *Midichloria mitochondrii* [72]. However, while low in abundance, male ticks still harbor *Lariskella*, and *Lariskella* sequences have been detected in males by both qPCR [33] and amplicon sequencing [28, 71]. In contrast, we failed to detect any *Lariskella* within males using diagnostic PCR or DOPE-FISH microscopy. Notably, the DOPE-FISH microscopy was sensitive enough to visualize individual *Lariskella* cells and still failed to detect *Lariskella* infection in males.

Our observed lack of male infection led us to cautiously hypothesize that *Lariskella* might be a reproductive

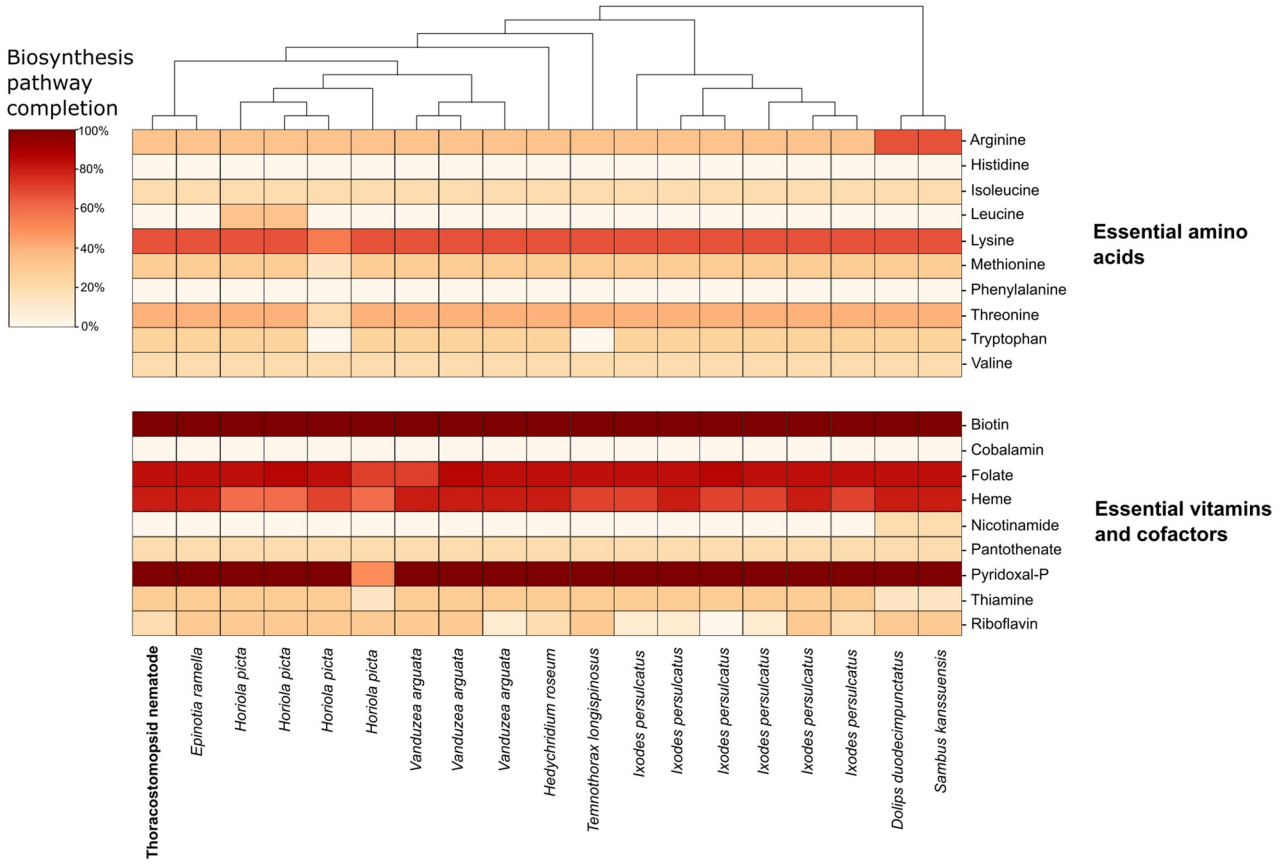


**Fig. 5** Heatmap displaying the completion of biosynthesis pathways in the two publicly available *Lariskella* genomes and *Lariskella* MAGs generated in this study. Genomes were clustered based on their phylogeny found in Fig. 1. Pathway completion was found by calculating KEGG module completion based on K-terms generated by eggnog-mapper annotation. The shown pathways correspond to nutrients essential to nematodes, based on findings in *Caenorhabditis elegans*

parasite in nematodes. *Lariskella* is a reproductive manipulator in insects, where it has recently been demonstrated to be capable of inducing cytoplasmic incompatibility [31]. However, cytoplasmic incompatibility relies on infected males, and our results suggest an absence of infected males in the nematode population. Instead, the infection pattern observed in nematodes appears indicative of a male-killing or feminizing effect, as this would explain both the lack of male infection and the lower male prevalence. Additionally, a feminizing or male-killing effect would be more in line with the observed infection rate. As mentioned above, *Lariskella* tends to occur at higher frequencies in arthropods, which is considered typical of symbionts inducing cytoplasmic incompatibility [73]. In contrast, feminizing or male-killing symbionts are typically maintained at lower frequencies [6], to allow for sufficient male births to sustain the population. Our observed female infection rate (26%) appears in line with those observed for feminizing symbionts (15%-42%) [74–76] or male-killing symbionts (< 40%) [6]. We also found potential orthologs of putative male-killing genes within the analyzed *Lariskella* genomes (Supplemental_sequences). The inferred orthology was found to be based on a shared Cro/C1-type helix-turn-helix DNA-binding domain, which is commonly encountered in putative male-killing genes (Supplemental Figure S7) [77–79]. However, attempts to culture the nematode have failed, and we thus lack the ability to experimentally test for male-killing or feminization.

### Expanded host range of Lariskella

The novel finding of a marine nematode host contrasts with the known host range of *Lariskella*, which is limited to terrestrial arthropods. To assess whether this novel host represents a wider pattern, we performed an exhaustive screening of the literature and the NCBI SRA database, compiling a comprehensive list of currently known *Lariskella* hosts (Supplemental Table S3). No other nematode or marine hosts were found. This might indicate that *Lariskella* infection of such hosts is rare. Alternatively, the failure to find *Lariskella* could be attributed to an undersampling of marine invertebrates, and particularly nematodes. Nematodes numbered an impressive 95,641 entries in the SRA database (on October 10th,

2025). However, the sampling represents only a fraction of nematode diversity, with only 10 species accounting for over 80% of all entries, and *Caenorhabditis elegans* representing over two thirds of all nematode entries.

Regardless of its prevalence, the capability to infect nematodes represents an interesting parallel between *Lariskella* and *Wolbachia*. *Wolbachia* is a group of highly studied Alphaproteobacteria famous for their success as endosymbionts, and it is estimated to infect 50% of all arthropods [80]. *Wolbachia* are known to infect arachnids and insects [80], as well as nematodes [81, 82], all of which are now known to also harbor *Lariskella* (Supplemental Figure S8). The nested placement of nematode-infecting *Lariskella* among insect-infecting strains also represents a parallel with *Wolbachia*, as several studies indicate that nematode-infecting *Wolbachia* supergroups are also nested among insect-infecting supergroups [83, 84]. It warrants mention that *Wolbachia* are also known to infect Isopoda [85], for which no *Lariskella* have been discovered to date. However, like nematodes, isopod diversity is undersampled (4735 SRA entries), and we suspect further sampling of isopods might reveal *Lariskella* infection, particularly as our findings illustrate the ability of *Lariskella* to infect marine hosts. If this is indeed true, it would raise interesting questions regarding the evolution of Rickettsiales and host choice, as *Wolbachia* and *Lariskella* are only distantly related, yet seem to have independently converged on a similar host range.

### Lariskella is not an obligate nutritional symbiont in nematodes

*Lariskella* has been hypothesized to play a nutritional role in ticks, acting as a potential source of B vitamins [29]. We confirmed the ability of *Lariskella* to synthesize several essential B vitamins, which could potentially support host nutrition. However, as *Lariskella* is absent in the majority of the nematode population, we can conclude that the nematode host does not rely on *Lariskella* to source essential B vitamins. The supplementation of B vitamins might instead be a method by which *Lariskella* could offset the fitness cost of infection, allowing it to spread in the host population more effectively, as has been shown in *Wolbachia* [86]. Though, notably *Lariskella* presence or absence has been shown to not affect the fitness of an insect host [31], suggesting *Lariskella* might not significantly contribute to nutrition.

## Conclusions

Here, we confirmed that *Lariskella* is an endosymbiont of a marine nematode species. This illustrates that *Lariskella* can infect nematodes and marine hosts, implying a vastly larger host range than currently described. However, screening of the SRA database did not reveal any further nematode or marine hosts. We attribute this to an undersampling of nematodes and marine invertebrates, although we cannot rule out the possibility that the infection in nematodes could be a rare exception.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-026-12881-x.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

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#### Authors' contributions

AH led the sampling, data analysis, and manuscript writing. YM aided in TEM microscopy, while PP assisted in the reproductive manipulation gene analyses. The principal investigator FH oversaw the project, and assisted in data interpretation and manuscript writing.

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#### Data availability

The nematode data used in this study are publicly available on NCBI as BioProject PRJNA1272136, SRA-derived *Lariskella* MAGs are available from FigShare: 10.6084/m9.figshare.30943895. Commands and scripts used for the analyses are available from [https://github.com/ECBSU/ECBSU_manuscripts_code/tree/main/Hagenbeek_nematode_***lariskella***].

## Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests.

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#### References

1. Ettema TJG, Andersson SGE. The α-proteobacteria: the Darwin finches of the bacterial world. Biol Lett. 2009;5:429–32.
2. Renvoisé A, Merhej V, Georgiades K, Raoult D. Intracellular Rickettsiales: Insights into manipulators of eukaryotic cells. Trends Mol Med. 2011;17:573–83.
3. Hosokawa T, Koga R, Kikuchi Y, Meng X-Y, Fukatsu T. Wolbachia as a bacteriocyte-associated nutritional mutualist. Proc Natl Acad Sci. 2010;107:769–74.
4. Zhao D, Zhang Z, Niu H, Guo H. Pathogens are an important driving force for the rapid spread of symbionts in an insect host. Nat Ecol Evol. 2023;7:1667–81.

5. Pannebakker BA, Loppin B, Elemans CPH, Humblot L, Vavre F. Parasitic inhibition of cell death facilitates symbiosis. Proc Natl Acad Sci U S A. 2007;104:213–5.
6. Engelstädter J, Hurst GDD. The Ecology and Evolution of Microbes that Manipulate Host Reproduction. Annu Rev Ecol Evol Syst. 2009;40:127–49.
7. Montagna M, Sassera D, Epis S, Bazzocchi C, Vannini C, Lo N, Sacchi L, Fukatsu T, Petroni G, Bandi C. Candidatus Midichloriaceae fam. nov. (Rickettsiales), an Ecologically Widespread Clade of Intracellular Alphaproteobacteria. Appl Environ Microbiol. 2013;79:3241–8.
8. Giannotti D, Boscaro V, Husnik F, Vannini C, Keeling PJ. The Other Rickettsiales: an Overview of the Family Candidatus Midichloriaceae. Appl Environ Microbiol. 2022;88:e02432–21.
9. Vannini C, Ferrantini F, Schleifer K-H, Ludwig W, Verni F, Petroni G. Candidatus Anadelfobacter veles and Candidatus Cyrtobacter comes, Two New Rickettsiales Species Hosted by the Protist Ciliate Euplotes harpa (Ciliophora, Spirotrichea). Appl Environ Microbiol. 2010;76:4047–54.
10. Szokoli F, Sabaneyeva E, Castelli M, Krenek S, Schrallhammer M, Soares CAG, da Silva-Neto ID, Berendonk TU, Petroni G. Candidatus Fokinia solitaria, a Novel Stand-Alone Symbiotic Lineage of Midichloriaceae (Rickettsiales). PLoS ONE. 2016;11:e0145743.
11. Schulz F, Martijn J, Wascher F, Lagkouvardos I, Kostanjšek R, Ettema TJG, Horn M. A Rickettsiales symbiont of amoebae with ancient features. Environ Microbiol. 2016;18:2326–42.
12. Muñoz-Gómez SA, Hess S, Burger G, Lang BF, Susko E, Slamovits CH, Roger AJ. An updated phylogeny of the Alphaproteobacteria reveals that the parasitic Rickettsiales and Holosporales have independent origins. eLife. 2019;8:e42535.
13. Driscoll T, Gillespie JJ, Nordberg EK, Azad AF, Sobral BW. Bacterial DNA Sifted from the Trichoplax adhaerens (Animalia: Placozoa) Genome Project Reveals a Putative Rickettsial Endosymbiont. Genome Biol Evol. 2013;5:621–45.
14. Kamm K, Osigus H-J, Stadler PF, DeSalle R, Schierwater B. Genome analyses of a placozoan rickettsial endosymbiont show a combination of mutualistic and parasitic traits. Sci Rep. 2019;9:17561.
15. Klinges JG, Rosales SM, McMinds R, Shaver EC, Shantz AA, Peters EC, Eitel M, Wörheide G, Sharp KH, Burkepile DE, Silliman BR, Vega Thurber RL. Phylogenetic, genomic, and biogeographic characterization of a novel and ubiquitous marine invertebrate-associated Rickettsiales parasite, Candidatus Aquarickettsia rohweri, gen. nov., sp. nov. ISME J. 2019;13:2938–53.
16. Mancini E, Sabatelli S, Hu Y, Frasca S, Di Giulio A, Audisio P, Brown CD, Russell JA, Trizzino M. Uncovering Active Bacterial Symbionts in Three Species of Pollen-feeding Beetles (Nitidulidae: Meligethinae). Microb Ecol. 2023;85:335–9.
17. Kurilshikov A, Livanova NN, Fomenko NV, Tupikin AE, Rar VA, Kabilov MR, Livanov SG, Tikunova NV. Comparative Metagenomic Profiling of Symbiotic Bacterial Communities Associated with Ixodes persulcatus, Ixodes pavlovskyi and Dermacentor reticulatus Ticks. PLoS ONE. 2015;10:e0131413.
18. Toju H, Tanabe AS, Notsu Y, Sota T, Fukatsu T. Diversification of endosymbiosis: replacements, co-speciation and promiscuity of bacteriocyte symbionts in weevils. ISME J. 2013;7:1378–90.
19. Morrow JL, Hall AAG, Riegler M. Symbionts in waiting: the dynamics of incipient endosymbiont complementation and replacement in minimal bacterial communities of psyllids. Microbiome. 2017;5:58.
20. Matsuura Y, Kikuchi Y, Meng XY, Koga R, Fukatsu T. Novel Clade of Alphaproteobacterial Endosymbionts Associated with Stinkbugs and Other Arthropods. Appl Environ Microbiol. 2012;78:4149–56.
21. Sassera D, Beninati T, Bandi C, Bouman EAP, Sacchi L, Fabbi M, Lo N. Candidatus Midichloria mitochondrii', an endosymbiont of the tick Ixodes ricinus with a unique intramitochondrial lifestyle. Int J Syst Evol Microbiol. 2006;56:2535–40.
22. Mediannikov OI, Ivanov LI, Nishikawa M, Saito R, Sidel'nikov IN, Zdanovskaia NI, Mokretsova EV, Tarasevich IV, Suzuki H. [Microorganism Montezuma of the order Rickettsiales: the potential causative agent of tick-borne disease in the Far East of Russia]. Zh Mikrobiol Epidemiol Immunobiol. 2024;1:7–13.
23. Bazzocchi C, Mariconti M, Sassera D, Rinaldi L, Martin E, Cringoli G, Urbanelli S, Genchi C, Bandi C, Epis S. Molecular and serological evidence for the circulation of the tick symbiont Midichloria (Rickettsiales: Midichloriaceae) in different mammalian species. Parasit Vectors. 2013;6:350.
24. Mariconti M, Epis S, Gaibani P, Dalla Valle C, Sassera D, Tomao P, Fabbi M, Castelli F, Marone P, Sambri V, Bazzocchi C, Bandi C. Humans parasitized by the hard tick Ixodes ricinus are seropositive to Midichloria mitochondrii: is Midichloria a novel pathogen, or just a marker of tick bite? Pathog Glob Health. 2012;106:391–6.
25. Mariconti M, Epis S, Sacchi L, Biggiogera M, Sassera D, Genchi M, Alberti E, Montagna M, Bandi C, Bazzocchi C. A study on the presence of flagella in the order Rickettsiales: the case of 'Candidatus Midichloria mitochondrii'. Microbiology. 2012;158:1677–83.
26. Sassera D, Lo N, Epis S, D'Auria G, Montagna M, Comandatore F, Horner D, Peretó J, Luciano AM, Franciosi F, Ferri E, Crotti E, Bazzocchi C, Daffonchio D, Sacchi L, Moya A, Latorre A, Bandi C. Phylogenomic Evidence for the Presence of a Flagellum and cbb3 Oxidase in the Free-Living Mitochondrial Ancestor. Mol Biol Evol. 2011;28:3285–96.
27. Grandi G, Chiappa G, Ullman K, Lindgren P-E, Olivieri E, Sassera D, Östlund E, Omazic A, Perissinotto D, Söderlund R. Characterization of the bacterial microbiome of Swedish ticks through 16S rRNA amplicon sequencing of whole ticks and of individual tick organs. Parasit Vectors. 2023;16:39.
28. Becker NS, Rollins RE, Stephens R, Sato K, Brachmann A, Nakao M, Kawabata H. Candidatus Lariskella arthopodarum endosymbiont is the main factor differentiating the microbiome communities of female and male Borrelia-positive Ixodes persulcatus ticks. Ticks Tick-Borne Dis. 2023;14:102183.
29. Buysse M, Duron O. Evidence that microbes identified as tick-borne pathogens are nutritional endosymbionts. Cell. 2021;184:2259–60.
30. Lu M, Meng C, Zhang B, Wang X, Tian J, Tang G, Wang W, Li N, Li M, Xu X, Sun Y, Duan C, Qin X, Li K. Prevalence of Spotted Fever Group Rickettsia and Candidatus Lariskella in Multiple Tick Species from Guizhou Province, China. Biomolecules. 2022;12(11):1701.
31. Umanzor EF, Kelly SE, Ravenscraft A, Matsuura Y, Hunter MS. The facultative intracellular symbiont Lariskella is neutral for lifetime fitness and spreads through cytoplasmic incompatibility in the leaffooted bug, Leptoglossus zonatus. bioRxiv. 2025. https://doi.org/10.1101/2025.01.29.635435.
32. Aivelo T, Tschirren B. Bacterial microbiota composition of a common ectoparasite of cavity-breeding birds, the Hen Flea Ceratophyllus gallinae. Ibis. 2020;162:1088–92.
33. Mukhacheva TA, Kovalev SY. Bacteria of the Family 'Candidatus Midichloriaceae' in Sympatric Zones of < em>Ixodes Ticks: Genetic Evidence for Vertical Transmission. Microb Ecol. 2017;74:185–93.
34. Madeira F, Madhusoodanan N, Lee J, Eusebi A, Niewielska A, Tivey ARN, Lopez R, Butcher S. The EMBL-EBI Job Dispatcher sequence analysis tools framework in 2024. Nucleic Acids Res. 2024;52:W521–5.
35. Chen S. Ultrafast one-pass FASTQ data preprocessing, quality control, and deduplication using fastp. iMeta. 2023;2:e107.
36. Li D, Liu C-M, Luo R, Sadakane K, Lam T-W. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics. 2015;31:1674–6.
37. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9:357–9.
38. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, Whitwham A, Keane T, McCarthy SA, Davies RM, Li H. Twelve years of SAMtools and BCFtools. GigaScience. 2021;10:giab008.
39. Kang DD, Li F, Kirton E, Thomas A, Egan R, An H, Wang Z. MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. PeerJ. 2019;7:e7359.
40. Alneberg J, Bjarnason BS, de Bruijn I, Schirmer M, Quick J, Ijaz UZ, Lahti L, Loman NJ, Andersson AF, Quince C. Binning metagenomic contigs by coverage and composition. Nat Methods. 2014;11:1144–6.
41. Pan S, Zhao X-M, Coelho LP. SemiBin2: self-supervised contrastive learning leads to better MAGs for short- and long-read sequencing. Bioinformatics. 2023;39:i21–9.
42. Sieber CMK, Probst AJ, Sharrar A, Thomas BC, Hess M, Tringe SG, Banfield JF. Recovery of genomes from metagenomes via a dereplication, aggregation and scoring strategy. Nat Microbiol. 2018;3:836–43.
43. Huson DH, Auch AF, Qi J, Schuster SC. MEGAN analysis of metagenomic data. Genome Res. 2007;17:377–86.
44. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. BLAST+: architecture and applications. BMC Bioinformatics. 2009;10:421.
45. Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, Salzberg SL. Versatile and open software for comparing large genomes. Genome Biol. 2004;5:R12.
46. Chklovski A, Parks DH, Woodcroft BJ, Tyson GW. CheckM2: a rapid, scalable and accurate tool for assessing microbial genome quality using machine learning. Nat Methods. 2023;20:1203–12.
47. Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH. GTDB-Tk v2: memory friendly classification with the genome taxonomy database. Bioinformatics. 2022;38:5315–6.

48. Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 2014;30:2068–9.
49. Emms DM, Kelly S. OrthoFinder: phylogenetic orthology inference for comparative genomics. Genome Biol. 2019;20:238.
50. Rozewicki J, Li S, Amada KM, Standley DM, Katoh K. MAFFT-DASH: integrated protein sequence and structural alignment. Nucleic Acids Res. 2019;47:W5–10.
51. Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics. 2009;25:1972–3.
52. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Mol Biol Evol. 2020;37:1530–4.
53. Gruber-Vodicka HR, Seah BKB, Pruesse E. phyloFlash: Rapid Small-Subunit rRNA Profiling and Targeted Assembly from Metagenomes. mSystems. 2020;5:e00920–20.
54. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41:D590–6.
55. van Megen E, Holterman M, Karssen G, Mooijman P, Bongers AMT, Holovachov O, Bakker J, Helder J. A phylogenetic tree of nematode based on about 1200 full-length small subunit ribosomal DNA sequences. Nematol. 2009;11(6):927–50.
56. Meldal BHM, Debenham NJ, De Ley P, De Ley IT, Vanfleteren JR, Vierstraete AR, Bert W, Borgonie G, Moens T, Tyler PA, Austen MC, Blaxter ML, Rogers AD, Lambshead PJD. An improved molecular phylogeny of the Nematoda with special emphasis on marine taxa. Mol Phylogenet Evol. 2007;42:622–36.
57. SILVA ribosomal RNA gene database project. : improved data processing and web-based tools | Nucleic Acids Research | Oxford Academic. https://academic.oup.com/nar/article/41/D1/D590/1069277. Retrieved 13 November 2024.
58. Jones P, Binns D, Chang H-Y, Fraser M, Li W, McAnulla C, McWilliam H, Maslen J, Mitchell A, Nuka G, Pesseat S, Quinn AF, Sangrador-Vegas A, Scheremetjew M, Yong S-Y, Lopez R, Hunter S. InterProScan 5: genome-scale protein function classification. Bioinformatics. 2014;30:1236–40.
59. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Žídek A, Potapenko A, Bridgland A, Meyer C, Kohl SAA, Ballard AJ, Cowie A, Romera-Paredes B, Nikolov S, Jain R, Adler J, Back T, Petersen S, Reiman D, Clancy E, Zielinski M, Steinegger M, Pacholska M, Berghammer T, Bodenstein S, Silver D, Vinyals O, Senior AW, Kavukcuoglu K, Kohli P, Hassabis D. Highly accurate protein structure prediction with AlphaFold. Nature. 2021;596:583–9.
60. Wang J, Youkharibache P, Zhang D, Lanczycki CJ, Geer RC, Madej T, Phan L, Ward M, Lu S, Marchler GH, Wang Y, Bryant SH, Geer LY, Marchler-Bauer A. iCn3D, a web-based 3D viewer for sharing 1D/2D/3D representations of biomolecular structures. Bioinformatics. 2020;36:131–5.
61. Cantalapiedra CP, Hernández-Plaza A, Letunic I, Bork P, Huerta-Cepas J. eggNOG-mapper v2: Functional Annotation, Orthology Assignments, and Domain Prediction at the Metagenomic Scale. Mol Biol Evol. 2021;38:5825–9.
62. Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat Commun. 2018;9:5114.
63. Zečić A, Dhondt I, Braeckman BP. The nutritional requirements of Caenorhabditis elegans. Genes Nutr. 2019;14:15.
64. Kanehisa M, Sato Y, Kawashima M. KEGG mapping tools for uncovering hidden features in biological data. Protein Sci. 2022;31:47–53.
65. Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M. KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res. 2016;44:D457–62.
66. Holterman M, Schratzberger M, Helder J. Nematodes as evolutionary commuters between marine, freshwater and terrestrial habitats. Biol J Linn Soc. 2019;128:756–67.
67. Jensen P. Feeding ecology of free-living aquatic nematodes. Mar Ecol Prog Ser. 1987;35:187–96.
68. Clec'h WL, Chevalier FD, Genty L, Bertaux J, Bouchon D, Sicard M. Cannibalism and Predation as Paths for Horizontal Passage of Wolbachia between Terrestrial Isopods. PLoS ONE. 2013;8:e60232.
69. Tian J, Liu J, Zhao H, Chen X, Geng X, Lu M, Li K. Molecular surveillance reveals a potential hotspot of tick-borne disease in Yakeshi City, Inner Mongolia. BMC Microbiol. 2023;23:359.
70. Aivelo T, Norberg A, Tschirren B. Bacterial microbiota composition of Ixodes ricinus ticks: the role of environmental variation, tick characteristics and microbial interactions. PeerJ. 2019;7:e8217.
71. Namina A, Kazarina A, Lazovska M, Akopjana S, Ulanova V, Kivrane A, Freimane L, Sadovska D, Kimsis J, Bormane A, Capligina V, Ranka R. 2023. Comparative Microbiome Analysis of Three Epidemiologically Important Tick Species in Latvia. Microorganisms. 2023;11(8):1970.
72. Daveu R, Laurence C, Bouju-Albert A, Sassera D, Plantard O. Symbiont dynamics during the blood meal of *Ixodes ricinus* nymphs differ according to their sex. Ticks Tick-Borne Dis. 2021;12:101707.
73. Engelstädter J, Telschow A. Cytoplasmic incompatibility and host population structure. Heredity. 2009;103:196–207.
74. Vandekerckhove T, Watteyne S, Bonne W, Vanacker D, Devaere S, Rumes B, Maelfait J-P, Gillis M, Swings J, Braig HR. Evolutionary trends in feminization and intersexuality in woodlice (Crustacea, Isopoda) infected with Wolbachia pipientis (alpha-Proteobacteria). Belg J Zool. 2003;133:61–9.
75. Kageyama D, Nishimura G, Hoshizaki S, Ishikawa Y. Feminizing Wolbachia in an insect, Ostrinia furnacalis (Lepidoptera: Crambidae). 6. Heredity. 2002;88:444–9.
76. Negri I, Pellecchia M, Mazzoglio Pj, Patetta A, Alma A. Feminizing Wolbachia in Zyginidia pullula (Insecta, Hemiptera), a leafhopper with an XX/X0 sex-determination system. Proc R Soc B Biol Sci. 2006;273:2409–16.
77. Arai H, Anbutsu H, Nishikawa Y, Kogawa M, Ishii K, Hosokawa M, Lin S-R, Ueda M, Nakai M, Kunimi Y, Harumoto T, Kageyama D, Takeyama H, Inoue MN. Combined actions of bacteriophage-encoded genes in Wolbachia-induced male lethality. iScience. 2023;26(6):106842.
78. Perlmutter JI, Bordenstein SR, Unckless RL, LePage DP, Metcalf JA, Hill T, Martinez J, Jiggins FM, Bordenstein SR. The phage gene wmk is a candidate for male killing by a bacterial endosymbiont. PLOS Pathog. 2019;15:e1007936.
79. Kiefer JST, Schmidt G, Krüsemer R, Kaltenpoth M, Engl T. Wolbachia causes cytoplasmic incompatibility but not male-killing in a grain pest beetle. Mol Ecol. 2022;31:6570–87.
80. Weinert LA, Araujo-Jnr EV, Ahmed MZ, Welch JJ. The incidence of bacterial endosymbionts in terrestrial arthropods. Proc R Soc B Biol Sci. 2015;282:20150249.
81. Foster J, Ganatra M, Kamal I, Ware J, Makarova K, Ivanova N, Bhattacharyya A, Kapatral V, Kumar S, Posfai J, Vincze T, Ingram J, Moran L, Lapidus A, Omelchenko M, Kyrpides N, Ghedin E, Wang S, Goltsman E, Joukov V, Ostrovskaya O, Tsukerman K, Mazur M, Comb D, Koonin E, Slatko B. The Wolbachia Genome of Brugia malayi: Endosymbiont Evolution within a Human Pathogenic Nematode. PLOS Biol. 2005;3:e121.
82. Haegeman A, Vanholme B, Jacob J, Vandekerckhove TTM, Claeys M, Borgonie G, Gheysen G. An endosymbiotic bacterium in a plant-parasitic nematode: Member of a new *Wolbachia* supergroup. Int J Parasitol. 2009;39:1045–54.
83. Gerth M, Gansauge M-T, Weigert A, Bleidorn C. Phylogenomic analyses uncover origin and spread of the Wolbachia pandemic. Nat Commun. 2014;5:5117.
84. Kaur R, Shropshire JD, Cross KL, Leigh B, Mansueto AJ, Stewart V, Bordenstein SR, Bordenstein SR. Living in the endosymbiotic world of Wolbachia: A centennial review. Cell Host Microbe. 2021;29:879–93.
85. Cordaux R, Pichon S, Hatira HBA, Doublet V, Grève P, Marcadé I, Braquart-Varnier C, Souty-Grosset C, Charfi-Cheikhrouha F, Bouchon D. Widespread Wolbachia infection in terrestrial isopods and other crustaceans. ZooKeys. 2012;176:123–31.
86. Moriyama M, Nikoh N, Hosokawa T, Fukatsu T. Riboflavin Provisioning Underlies Wolbachia's Fitness Contribution to Its Insect Host. mBio. 2015;6. https://doi.org/10.1128/mbio.01732-15.


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