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Jiella semarangensis sp. nov., isolated from mangrove sediment in Semarang city, Indonesia

Mada Triandala Sibero
Chinese Academy of Medical Sciences & Peking Union Medical College
Zhou-Qing Zheng
Zunyi Medical University
Fei-Na Li
Capital Medical University
Shao-Wei Liu
Chinese Academy of Medical Sciences & Peking Union Medical College
Chengshao Zan
Zunyi Medical University
Xin Xiang
Qinghai University
Li Tuo
Zunyi Medical University
Cheng-Hang Sun
chenghangsun@hotmail.com

Chinese Academy of Medical Sciences & Peking Union Medical College

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Abstract

A novel Gram-stain-negative, aerobic, and rod-shaped bacterium strain M17.18^T, was isolated from mangrove sediment collected from Semarang City, Central Java Province, Indonesia. Phylogenetic analysis based on almost complete 16S rRNA gene sequences indicated that this strain belonged to the genus *Jiella*, with the highest similarity to *Jiella mangrovi* KSK16Y-1^T (98.5%), *Jiella flava* CQZ9-1^T (97.7%), *Jiella sonneratiae* MQZ13P-4^T (97.6%), *Jiella endophytica* CBS5Q-3^T (97.4%) and *Jiella aquimaris* LZB041^T (97.1%). The whole-genome sequence of strain M17.18T was 4.93 Mb, with a 67.8% G + C content. The whole genome comparison between strain M17.18^T with *J. mangrovi* KSK16Y-1^T, *J. flava* CQZ9-1^T, and *J. aquimaris* 22II-16-19i^T for average nucleotide identity (ANI) values (< 79.3%) and digital DNA-DNA hybridization (dDDH) values (< 22.9%), confirmed a low genome relatedness. Strain M17.18^T grew at 4–45°C (optimum, 30–37°C), pH 4–8 (optimum, 6–7), with salinity 0–13% (optimum, 1–2%). Ubiquinone-10 (Q-10) was recorded as the predominant respiratory quinone. The $C_{18:1}\omega7c$ and $C_{19:0}$cycloω8c were the major fatty acids. The polar lipid profile consisted of phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, diphosphatidylglycerol, one unknown aminophospholipid, one unknown aminolipid, three unknown phospholipids, and six unknown lipids. The polyphasic characterization leaded strain M17.18^T as a novel *Jiella* species. The name *Jiella semarangensis* sp. nov., type strain M17.18^T (= KCKC 8278^T = MCCC 1K08882^T) is proposed.

Introduction

For the past decade, *Jiella* has been noted as one of the novel genera from family *Aurantimonadaceae*, class *Alphaproteobacteria* and order *Hyphomicrobiales* [1]. This genus was initially described in 2015 by Liang *et al*. [2] with *Jiella aquimaris* JCM 30119^T as the type strain. In total, 10 species with validly published names have been successfully described from this genus (https://lpsn.dsmz.de/genus/jiella). It is highlighted that all members of *Jiella* were isolated from marine and coastal environments, including water [2–4], sediment [5], and living as an endophyte in mangroves [5–9]. Moreover, most members of *Jiella* are also noted as aerobic bacterium, motile, possess ubiquinone-10 as the predominant respiratory quinone, with $C_{18:1}\omega7c$ as the major fatty acid [2, 5, 7, 8].

As a tropical maritime country, Indonesia nurtures plenty of mangrove ecosystems that harbour an enormous number of novel bacterial candidates. Numerous studies have demonstrated that mangrove-associated bacteria from Indonesia have the potential as bioactive producers [10–12]. During the investigation of bacterial biodiversity in Indonesia, a novel bacterium, designated as M17.18^T was isolated from mangrove sediment. The 16S rRNA gene sequence of strain M17.18^T exhibited a low similarity score to the type strain of *Jiella mangrovi* KSK16Y-1^T (98.5%), *Jiella flava* CQZ9-1^T (97.7%), *Jiella sonneratiae* MQZ13P-4^T (97.6%), *Jiella endophytica* CBS5Q-3^T (97.4%) and *Jiella aquimaris* LZB041^T

(97.1%). This current study aimed to provide an exact taxonomic status of strain M17.18^T by using a polyphasic taxonomic approach.

Materials and Methods

Isolation and Cultivation

Mangrove sediment for isolation of strain M17.18^T was collected from Semarang City, Central Java, Indonesia (06° 57' 274" S 110 °18' 851" E). The sediment was taken from a depth of 10–15 cm around the root of *Rhizophora apiculata*. Then, the sample was air-dried using flowing air inside of laminar flow hood. Afterwards, approximately 1.0 g of the sample was diluted in 100 mL of sterile normal saline. In total, 100 µL of the mixture was spread on an agar medium, which consisted of glycerine (15 g L^{-1}), L-tyrosine (0.5 g L^{-1}), L-asparagine (1 g L^{-1}), K_2HPO_4 (0.5 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.5 g L^{-1}), NaCl (0.5 g L^{-1}), trace salt solution (1 mL L^{-1}), agar (15 g L^{-1}) with pH 6.0. The recipe for trace salt solution is as below: $FeSO_4 \cdot 7H_2O$ (0.2 g), $MnCl_2 \cdot 4H_2O$ (0.01 g), $ZnSO_4 \cdot 7H_2O$ (0.01 g) in 100 mL distilled water [14]. After incubation at 30°C for 14 days, colonies of strain M17.18^T appeared then purified on the International *Streptomyces* Project (ISP) 2 agar medium with the addition of 1% NaCl with serial streaking technique. The pure colony was maintained on the ISP 2 agar with 1% NaCl and pH 7.0 and then preserved in 20% glycerol at -80°C for further use.

Phylogenetic Analysis Based on 16S rRNA Gene Sequence

A Chelex-100 (5%, w/v) solution (Bio-Rad Laboratories, Inc.) was used to obtain genomic DNA from strain M17.18^T, and then the 16S rRNA gene sequence was amplified with 27F and 1492R using a PCR thermal cycler [15]. Furthermore, the purified PCR products were cloned using a pEASY-T1 cloning kit (TransGen Biotech) according to the manufacturer's protocols to obtain an almost full 16S rRNA gene sequence. The EzBioCloud server (https://www.ezbiocloud.net/) was used to identify related species using the Nucleotide Similarity Search program based on the almost complete 16S rRNA gene sequence [15–16]. Phylogenetic trees were reconstructed using neighbour-joining [18], maximum likelihood [19], and maximum-parsimony [20] algorithms in the Molecular Evolutionary Genetics Analysis (MEGA) software package version X [21]. Kimura's two-parameter model was applied to calculate the evolutionary distance [22], while the topologies of the phylogenetic trees were evaluated using bootstrap analysis with 1000 repetitions [23].

Genome Sequencing and Annotation

A DNA extraction kit from Shanghai Majorbio Bio-Pharm Technology Co. Ltd. was applied to obtain the genomic DNA of strain M17.18^T. Draft-genome sequencing was done using the Illumina Novaseq 6000 platform. The A-tailed, ligated to paired-end adaptors, and PCR amplified with a ~ 400 bp were used to

prepare library reconstruction at Shanghai Majorbio Bio-Pharm Technology Co. Ltd. (www.majorbio.com). Raw reads of each data set were filtered using fastp software (version 0.19.6) [24], then assembled using SOPA *de novo* version 2.04 [25]. Subsequently, gap-closing was performed on the filter reads. Genome annotation and analysis were carried out using SEED, Rapid Annotation Subsystem Technology (RAST) web server (http://rast.nmpdr.org/) [25–27]. The Bacterial and Viral Bioinformatics Resources Center (BV-BRC) (https://www.bv-brc.org/) was also used for genome annotation and reconstructing a circular genomic map [29]. In addition, the biosynthetic gene cluster (BGC) was annotated using antiSMASH version 7.0 [30].

Genome Comparison and Phylogenomic Analysis

All genomes under the genus *Jiella*, including the three reference strains, namely *J. mangrovi* KSK16Y-1^T, *J. flava* CQZ9-1^T, and *J. aquimaris* 22II-16-19i were downloaded from the GenBank database (https://www.ncbi.nlm.nih.gov/datasets/genome/?taxon=1775688). Afterward, the average nucleotide identity (ANI) value between strain M17.18^T and other related species from the *Jiella* genus was calculated using the ANI calculator in the EzBioCloud (https://www.ezbiocloud.net/tools/ani) [17]. A heatmap to visualize the ANI values was constructed using OAT software [31]. Then, the Genome-to-Genome Distance Calculator (GGDC 3.0) was used to calculate the digital DNA-DNA hybridization (dDDH) values [31–32].

Draft genome files of strain M17.18^T, *J. mangrovi* KSK16Y-1^T, *J. flava* CQZ9-1^T, *J. sonneratiae* MQZ13P-4^T, *J. endophytica* CBS5Q-3^T, *J. aquimaris* 22II-16-19i, and 16 related strains were uploaded to the automated multi-locus species tree (autoMLST) pipeline (https://automlst.ziemertlab.com/) to align the conserved core protein sequence from multiple loci within closely related genomes [34]. Afterwards, a phylogenetic tree from autoMLST alignment file was reconstructed using version X of the MEGA software package [21]. This phylogenetic tree reconstruction applied a maximum-likelihood method [35] and bootstrap analysis with 1000 repetitions [23].

The OrthoVenn3 platform (https://orthovenn3.bioinfotoolkits.net/) was applied to visualize the orthologous gene clusters [36]. In total, nine genomes consisting of species within the genus *Jiella* with the addition of some related species from NCBI Datasets (https://www.ncbi.nlm.nih.gov/datasets/genome/) were used for pangenome analysis using Anvi'o version 8.1 software [37]. Furthermore, the PanExplorer platform [38] was used to annotate strain-specific genes in strain M17.18^T.

Phenotypic Characteristics

Phenotypic profiling was conducted for strain M17.18^T, *J. mangrovi* KSK16Y-1^T, *J. flava* CQZ9-1^T, and *J. aquimaris* JCM 30119^T by culturing them on marine agar (MA; BD), ISP 2 agar, tryptic soy agar (TSA; BD), nutrient agar (NA; BD), R2A agar (BD), brain heart infusion agar (BHI; Hopebio), and Luria-Bertani agar

(LB; Solarbio) for 2 weeks at 30°C. Colonial growth, color, and soluble pigment were compared during the growth. Cell morphology of 5- day-old strain M17.18^T on MA was observed using transmission electron microscopy (JEM-1400, JEOL). The motility of this strain was evaluated using Sulphide Indole Motility (SIM) agar after 3 days at 30°C, while Gram-staining was applied according to Magee *et al*. [39]. The ability of strain M17.18^T to grow under anaerobic conditions was observed in the AnaeroPack™ Anaerobic System (MGC) for 14 days at 30°C. Bacterial growth was evaluated on MA over a period of 14 days at various temperature points: 4, 20, 28, 30, 37, 40, and 45°C. Then, the pH range for growth was determined on MA with a pH range of 5–12 (intervals of 1.0 pH unit) for 14 days at 30°C using the buffer system described by Xu *et al*. [40]. Medium ISP 2 agar was used to observe the salt tolerance by adding 0–13% NaCl (w/v) (intervals of 1% unit) for 14 days at 30°C. Catalase activity test was done by adding several drops of H_2O_2 (2%, v/v); the formation of air bubbles indicates a positive result. Whereas the oxidase activity was tested using an oxidase reagent kit from bioMérieux. The ability of acid production using different carbon sources was detected using API 50CH (bioMérieux, France), while enzyme activity was observed using API ZYM (bioMérieux, France). More biochemical profiles were also determined using API 20NE (bioMérieux, France). Additionally, the ability to hydrolyze macromolecules, including starch, cellulose, milk, gelatine, and Tweens 20, 40, and 80, was evaluated using the agar plates test [41].

Chemotaxonomic Characterization

Strain M17.18^T, *J. mangrovi* KSK16Y-1^T, *J. flava* CQZ9-1^T, and *J. aquimaris* JCM 30119^T were cultivated until reaching the late-exponential phase on MA for 3 days at 28°C to obtain biomass for whole-cell fatty acids characterization. A standard method, including saponification, methylation, extraction, and washing steps using alkali, was applied to obtain fatty acids [42]. The composition of fatty acids was injected into a Thermo Scientific™ TRACE™ 1310 Gas-Chromatograph coupled with a Thermo Scientific™ ISQ™ QD single quadruple mass spectrometer, and then the NIST08 Library database was used to identify the type of fatty acids [43]. Furthermore, ISP2 agar with 2% NaCl was applied to grow strain M17.18^T for 4 days at 28°C. The biomasses were freeze-dried to obtain polar lipids and respiratory quinone. A protocol from Minnikin *et al*. [44] was applied to extract and purify respiratory quinone and polar lipids. Respiratory quinone identification was conducted using an HPLC coupled with a single quadrupole mass spectrometer (LCMS-2020; Shimadzu) [45]. Subsequently, profiling of polar lipids was conducted according to Tuo *et al*. [43] using two-dimensional TLC on a silica gel 60 F_{254} plates (Merck).

Results and Discussion

Phylogenetic Analysis Based on 16S rRNA Gene Sequence

Strain M17.18^T has 1485 bp for the almost complete 16S rRNA gene sequence. The result of species identification through the EzBioCloud server exhibited strain M17.18^T shared high similarity to *J. mangrovi* KSK16Y-1^T (98.5%), followed by *J. flava* CQZ9-1^T (97.7%), *J. sonneratiae* MQZ13P-4^T (97.6%), *J. endophytica* CBS5Q-3^T (97.4%) and *J. aquimaris* JCM LZB041^T (97.1%). The reconstructed phylogenetic

trees using the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms indicated that strain M17.18^T formed a monophyletic cluster with the members of the genus *Jiella* (Fig. 1, Supplementary Fig. S1 and S2), particularly concerning the placement of strain M17.18^T within the *Jiella* genus. It is also highlighted that strain M17.18^T was in one clade with *J. mangrovi* KSK16Y-1^T and *J. flava* CQZ9-1^T (Fig. 1), but the bootstrap value was notably low (< 70%). This suggests an unstable phylogenetic topology within the clade. Therefore, the position of strain M17.18^T should be determined using a polyphasic approach. In addition, *J. mangrovi* KSK16Y-1^T, *J. flava* CQZ9-1^T, and *J. aquimaris* JCM 30119^T (= LZB041^T) from the Japan Collection of Microorganisms (JCM) were used as the reference strains.

Genome Sequencing and Annotation

The assembled genome of strain M17.18^T had a genome size of 4.93 Mb, 58 contigs, 67.8% of G + C content, and an N50 value of 301631 bp. A total of 4926 predicted genes were annotated, including 4877 protein-coding sequences (CDS), 46 tRNA genes, and 3 rRNA genes. Additionally, Supplementary Fig. 3 displays the BV-BRC annotation results for strain M17.18^T which indicate the presence of 1791 hypothetical proteins, 3086 proteins with functional assignments, 1048 proteins with Enzyme Commission (EC) numbers [46], 907 proteins were assigned to gene ontology (GO) categories [47], and 815 proteins mapped to KEGG pathways [48]. Furthermore, the RAST platform successfully annotated 1132 coding genes across 325 subsystems, achieving 24% subsystem coverage. It is worth mentioning that the highest number of genes were assigned for amino acids and derivatives (295), followed by carbohydrate metabolisms (248), protein metabolism (193), cofactors, vitamins, prosthetic group, and pigments (128), respiration (121), fatty acids, lipids, and isoprenoids (99), nucleosides and nucleotides (85), membrane transport (74), DNA metabolism (70), and stress response (64).

Furthermore, the 64 genes associated with stress response consist of 26 genes responsible for oxidative stress, 12 genes responsible for osmotic stress, 2 genes responsible for detoxification, 2 genes responsible for periplasmic stress, and 3 genes in no subcategory. In addition, 12 genes that function for osmotic stress belong to 3 subsystems related to osmoregulation, choline and betaine uptake, betaine biosynthesis, and synthesis of osmoregulated periplasmic glucans. Furthermore, this strain harbours several interesting genes encoding choline and betaine uptake and biosynthesis. Glycine betaine is an essential osmoprotectant for bacteria to tolerate the hyper-salinity condition in their habitat. The interaction of glycine betaine and the modified extracellular polymeric substances helps bacteria increase their salinity tolerance [49]. Three subsystems that are responsible for the synthesis of osmoregulated periplasmic glucans are found in this strain. Proteobacteria commonly control the production of osmoregulated periplasmic glucans to overcome osmotic stress [50]. It was further explained that some bacteria will increase the production of this compound to maintain the cell morphology as the osmolarity in the medium decreases [51]. Still related to osmoregulation, one subsystem associated with the synthesis of Aquaporin Z is also found in strain M17.18^T. Aquaporin Z is a water channel protein that enables bacteria to control water flow as a response to the alteration of

cellular osmolarity [52]. The presence of these subsystems firmly explains the survival ability of strain M17.18^T in the mangrove ecosystem.

In order to investigate the biotechnological prospect, a number of biosynthetic gene clusters (BGCs), xenobiotic degradation and metabolisms were discovered in strain M17.18^T through BV-BRC and antiSMASH. Notably, one of the gene clusters was found to encode for a terpene, specifically associated with the production of pigment zeaxanthin with a similarity of 100%. Zeaxanthin is a carotenoid derivative characterized by hydroxyl groups at both terminal ends of its molecular structure and gives the colony a yellow to orange color [53]. The bright yellow colony color observed in strain M17.18^T can be attributed to the presence of this pigment, highlighting its significant role in this characteristic (Fig. 2a). It is highlighted that all members of the genus *Jiella* also possess a gene cluster responsible for producing carotenoids. Therefore, they also have a yellow-to-orange color [2–8]. The role of carotenoid-derived pigments, especially zeaxanthin, in non-photosynthetic bacteria is still not fully understood. However, some experts propose that this pigment may play a significant role in helping bacteria adapt to various environmental stresses, including exposure to ionizing and visible radiation, high temperatures, and varying salinity levels which are related to the osmosis process [53–56]. Furthermore, annotation using BV-BRC discovered some genes that are responsible for the degradation of gamma-hexachlorocyclohexane, bisphenol A (BPA), and 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT). Therefore, strain M17.18^T is also expected to have potential as a bioremediation agent.

Genome Comparison and Phylogenomic Analysis

The result of the ANI value calculation using OAT server revealed that among the three reference strains, strain M17.18^T shares the highest ANI value with *J. aquimaris* 22II-16-19i (78.8%), followed by *J. mangrovi* KSK16Y-1^T (77.8%), and *J. flava* CQZ9-1^T (77.0%) (Supplementary Fig. S4). Hereinafter, the dDDH values between strain M17.18^T and *J. mangrovi* KSK16Y-1^T, strain M17.18^T and *J. aquimaris* 22II-16-19i, as well as strain M17.18^T and *J. flava* CQZ9-1^T are 22.3%, 22.9%, and 21.9%, respectively. In the latest update of the minimal standard for identifying a novel species based on genomic data, Riesco and Trujillo [58] stated that the ANI value must be less than 95–96%, while the dDDH value must be less than 70%. It is highlighted that the ANI and dDDH values of strain M17.18^T, compared to its related reference strains, are lower than the threshold. This indicates that strain M17.18^T delineates a novel species from the genus *Jiella*.

The autoMLST pipeline effectively reconstructed a phylogenetic tree based on their 86 core genes in the genome of strain M17.18^T, as well as some related type strains. Notably, strain M17.18^T created a separated but stable clade alongside other type species of the genus *Jiella*, demonstrating a high bootstrap value (100%) (Fig. 3). Hence, this discovery further solidifies the taxonomic placement of strain M17.18^T within the genus *Jiella*. Furthermore, a comparative analysis of the genomic features between strain M17.18^T and the three closely related species is shown in Table 1. Thus, the genomic

comparison and phylogenetic analysis suggest that strain M17.18^T constitutes a novel species within the genus *Jiella*.

The orthologous gene cluster comparison using the OrthoVenn3 platform reveals a total of 4353 ortholog clusters within strain M17.18^T, *J. mangrovi* KSK16Y-1^T, *J. aquimaris* 22II-16-19i, and *J. flava* CQZ9-1^T. Then, 2397 ortholog clusters are noted as a shared homologous gene cluster, suggesting their conserved core genes within the genus. Notably, 52 unique gene clusters are found in strain M17.18^T, 29 gene clusters in *J. mangrovi* KSK16Y-1^T, 27 gene clusters in *J. flava* CQZ9-1^T, and 129 gene clusters in *J. aquimaris* 22II-16-19i (Supplementary Fig. S5). The result of pangenome analysis is shown in Fig. 4. It uttered 43,776 genes organized into 12,647 distinct gene clusters with 2,051 core genes, highlighting the complex genetic diversity that exists across the 20 genomes examined. Thereafter, this study also identified 6,603 unique genes exhibiting remarkable variation that define each genome, along with 3,993 dispensable genes. We found four unique gene clusters for diguanylate cyclase, one unique gene cluster for polysaccharide biosynthesis, and one unique gene cluster for tyrosine autokinase biosynthesis in strain M17.18^T through pangenome analysis using the PanExplorer platform, emphasizing the ability to develop exopolysaccharides to form biofilms [58–59]. Bacteria often produce biofilm to colonize nutrient-rich surfaces, like plant roots or mineral clay, as well as protect themselves from unfavourable environments due to temperature, salinity, pH, pressure, nutrient scarcity, including antibiotics [60–61].

Phenotypic Characteristics

Cells of strain M17.18^T were Gram-stain-negative, aerobic, motile with flagella, and rod-shaped (0.6–1.0 × 1.5–2.3 µm) (Fig. 2c, d). The colonies have a yellow color with a round shape, entire margin, and convex elevation on MA after 7 days of incubation at 30°C (Fig. 2a, b). Strain M17.18^T grew very well on MA, TSA, LB, and R2A, slightly slower on NA, less on ISP 2 agar, and poorly on BHI agar after 7 days of incubation at 30°C. It is worth mentioning that it did not produce aerial mycelia or soluble pigment. The colonies grew at 4–45°C (optimum at 30–37°C), pH at 4–8 (optimum pH 6–7), and concentration of NaCl of 0–13% (optimum 1–2%). In addition, this strain merely gave a weak capability to hydrolyze milk but was positive in hydrolyzing Tween 40, oxidase, and catalase. The API 20NE Kit strips gave positive results for the ability of strain M17.18^T to convert NO_3 to NO_2, urease, *β*-glucosidase, L-arabinose (weak), D-mannitol, *N*-acetylglucosamine, D-maltose (weak), potassium gluconate, adipic acid (weak), and malic acid (weak). Strain M17.18^T also gave positive results in API ZYM Kit strips for *β*-glucosidase, *β*-galactosidase, *N*-acetyl-*β*-glucosaminidase, *α*-mannosidase, cystine arylamidase, esterase lipase, *α*-glucosidase, valine arylamidase, and *α*-chymotrypsin. Additionally, API 50CH Kit strip exhibited the ability of this strain to metabolize D-arabinose, L-arabinose, D-xylose, methyl-*β* D-xylopyranoside, D-glucose, D-fructose, esculine ferric citrate, D-lyxose, D-fucose, L-fucose, and D-arabitol. Table 2 provides a complete phenotypic and biochemical profile distinguishing strain M17.18^T from the three reference strains.

Table 2

Phenotypic and biochemical profiles that distinguish the strain M17.18^T from its closely related species

Characteristics	**1**	**2**	**3**	**4**
Country	Indonesia	China	China	China
Isolation source	Mangrove sediment	Leaf of *Rhizophora stylosa*	Semi-mangrove plant *Acrostichum aureum*	Surface seawater
Motility	+	–	–	+
Catalase	+	+	+	+
Oxidase	+	+	+	+
Growth temperature range (°C)				
Range	4–45	4–40	4–40	4–40
Optimum	30–37	30–37	30–37	30–37
NaCl tolerance (%, w/v)				
Range	0–13	0–13	0–10	0–13
Optimum	1–2	0–1	0–1	0–1
pH range for growth				
Range	4–8	4–9	4–9	5–10
Optimum	6–7	6–7	6–7	6–7
Macromolecule hydrolyzation				
Tween–40	+	+	+	+
Milk	*w*	+	*w*	*w*
Enzyme activity (API ZYM)				
β-Glucosidase	+	+	+	+
β-Galactosidase	+	+	+	+
N-acetyl-*β*-glucosaminidase	+	+	+	+

Strains: 1, strain M17.18^T; 2, *J. mangrovi* KSK16Y-1^T; 3, *J. flava* CQZ9-1^T; 4, *J. aquimaris* JCM 30119^T. +, positive; -, negative; *w*, weak.

Characteristics	1	2	3	4
α-Mannosidase	+	+	+	+
Cystine arylamidase	+	+	+	+
Esterase lipase (C8)	+	+	+	+
α-Glucosidase	+	+	+	+
Valine arylamidase	+	+	+	+
α-Chymotrypsin	+	+	+	+
Carbon source utilization (API 20NE)				
NO_3 to NO_2	+	+	–	–
Glucose acidification	–	–	–	*w*
Urease	+	+	*w*	+
Esculin ferric citrate	+	+	*+*	+
D-Glucose	+	–	–	*w*
L-Arabinose	*w*	–	*w*	+
D-Mannose	–	+	+	*w*
D-Mannitol	+	+	+	*w*
N-acethyl-glucosamine	+	–	–	–
D-Maltose	*w*	+	*w*	*w*
Potassium gluconate	+	+	+	*w*
Adipic acid	*w*	+	*w*	*w*
Malate	*w*	*w*	*w*	+
Trisodium citrate	–	–	+	*w*

Strains: 1, strain M17.18^T; 2, *J. mangrovi* KSK16Y-1^T; 3, *J. flava* CQZ9-1^T; 4, *J. aquimaris* JCM 30119^T. +, positive; -, negative; *w*, weak.

Chemotaxonomic Characterization

The fatty acid profile of strain M17.18^T was dominated by $C_{18:1}\omega7c$ (68.7%), followed by $C_{19:0}$cyclo$\omega8c$ (19.0%), $C_{16:0}$ (8.4%), $C_{18:0}$ (3.4%), and $C_{12:0}$ (0.6%). The fact that $C_{18:1}\omega7c$ and $C_{19:0}$ cyclo $\omega8c$ were the two predominant cellular fatty acids in strain M17.18^T aligned with *J. mangrovi* KSK16Y-1^T, *J. flava* CQZ9-1^T and *J. aquimaris* JCM 30119^T. Interestingly, $C_{17:0}$, $C_{17:1}\omega6c$, and $C_{17:1}\omega8c$ were not found in

strain M17.18^T and *J. aquimaris* JCM 30119^T, yet these fatty acids were successfully detected and quantified in *J. mangrovi* KSK16Y-1^T and *J. flava* CQZ9-1^T, as described in Table 3. It is highlighted that Q-10 (98.9%) and Q-9 (1.1%) were detected in strain M17.18^T. The polar lipid in strain M17.18^T consisted of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylmonomethylethanolamine (PME), phosphatidylethanolamine (PE), six unknown lipids (L), three unknown phospholipids (PL), one unknown aminolipid (AL), and one unknown aminophospholipid (APL) (Supplementary Fig S6). This result exhibits that lipid composition in strain M17.18^T is similar to that of almost all species in the genus *Jiella*, which contain DPG, PG, PC, and PME.

Table 3

Cellular fatty acid composition (%) of strain M17.18^T and its closely related species

Fatty Acid	**1**	**2**	**3**	**4**
Straight Chain				
$C_{12:0}$	0.6	0.7	0.8	0.8
$C_{16:0}$	8.4	6.9	10.8	5.5
$C_{17:0}$	ND	1.4	1.9	ND
$C_{18:0}$	3.4	2.8	4.3	2.0
Unsaturated				
$C_{17:1}$ω6c	ND	0.8	0.6	ND
$C_{17:1}$ω8c	ND	TR	0.6	ND
$C_{18:1}$ω7c	68.7	68.1	61.4	39.7
$C_{19:0}$cycloω8c	19.0	18.9	19.6	52.0

Strains: 1, strain M17.18^T; 2, *J. mangrovi* KSK16Y-1^T; 3, *J. flava* CQZ9-1^T; 4, *J. aquimaris* JCM 30119^T. ND, not detected; TR, trace amount (< 0.5%).

Conclusion

Based on its genomic, phylogenetic, phenotypic, and chemotaxonomic characteristics, we formally propose that strain M17.18^T is considered a novel species of the genus *Jiella*, for which the name *Jiella semarangensis* sp. nov.is proposed.

Description of Jiella semarangensis sp. nov.

Jiella semarangensis (se.ma.rang.en'sis. N.L. masc. adj. *semarangensis*, pertaining to Semarang city, where the mangrove sediment was collected).

Cells are Gram-stain-negative, aerobic, motile with flagella, and rod-shaped (0.6–1.0 × 1.5–2.3 μm). Colonies are yellow with a round shape, entire margin, and convex elevation on MA after 7 days of incubation at 30°C. Grows very well on MA, TSA, LB, and R2A, slightly slower on NA, less on ISP 2 agar, and poorly on BHI agar after 7 days of incubation at 30°C. Growth conditions are 4–45°C (optimum at 30–37°C), pH at 4–8 (optimum pH 6–7), and concentration of NaCl of 0–13% (optimum 1–2%). Capable of hydrolysing milk and Tween 40, oxidase-positive, and catalase-positive. The API 20NE Kit strips exhibit its ability to reduce NO_3 to NO_2, produce urease, and hydrolize esculin. Has the ability to utilize D-glucose, D-mannitol, N-acetyl-glucosamine, and potassium gluconate as carbon sources. A weak utilization was observed for L-arabinose, D-maltose, adipic acid, and malic acid. The strain did not acidify glucose and did not utilize D-mannose or trisodium citrate. In the API ZYM Kit strips indicate the ability of the strain to produce *β*-glucosidase, *β*-galactosidase, N-acetyl-*β*-glucosaminidase, *α*-mannosidase, cystine arylamidase, esterase lipase, *α*-glucosidase, valine arylamidase, and *α*-chymotrypsin. Furthermore, the API 50CH Kit strip shows the ability of this strain to metabolize D-arabinose, L-arabinose, D-xylose, methyl-*β* D-xylopyranoside, D-glucose, D-fructose, esculine ferric citrate, D-lyxose, D-fucose, L-fucose, and D-arabitol. The dominant respiratory quinone is Q-10. The polar lipid consists of DPG, PG, PC, PME, PE, L 1–6, PL 1–3, AL, and APL. The major fatty acids are $C_{18:1}\omega7c$, and $C_{19:0}$cyclo$\omega8c$.

The type strain M17.18^T (= KCTC 8278^T = MCCC 1K08882^T) was isolated from mangrove sediment in Semarang city, Central Java Province, Indonesia. The total draft genome sequence was 4.93Mb, with 67.8% G + C content. The 16S rRNA gene and draft whole genome sequences of strain M17.18^T have been deposited in GenBank with accession numbers PQ037108 and JBFTEH000000000, respectively.

Abbreviations

ANI, average nucleotide identity; AutoMLST, automated multi-locus species tree; BHI, brain–heart infusion; dDDH, digital DNA–DNA hybridization; ISP, International *Streptomyces* Project; LB, Luria-Bertani agar; MA, marine agar; NA, nutrient agar; RAST, Rapid Annotation Subsystem Technology; TSA, tryptic soy agar.

Declarations

Author Contributions

MTS: conceptualisation, microbial isolation, data collection, data analysis, manuscript writing. **ZQZ:** data collection, data analysis, and manuscript writing. **FNL:** respiratory quinone analysis, data analysis, and manuscript writing. **SWL:** conceptualisation, data analysis, manuscript writing. **CZ:** PanGenomic analysis and manuscript writing. **XX:** Genomic and bioinformatic analysis. **LT:** conceptualisation, design of the research, supervision of the experiment, data curation, data analysis, manuscript writing. **CHS:** secure the funding, conceptualisation, design of the research, supervision of the experiment, and manuscript writing. All authors have read and approved the final manuscript.

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Data availability

The GenBank accession numbers for the 16S rRNA gene sequences and the whole genome sequence of *Jiella semarangensis* sp. nov. MT17.18^T are PQ037108 and JBFTEH010000000, respectively. The BioProject ID PRJNA1140834 containing BioSample SAMN42857473.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Ethical Statement

This article does not contain any studies with human participants or animals.

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Table 1

Table 1 is available in the Supplementary Files section.

Figures

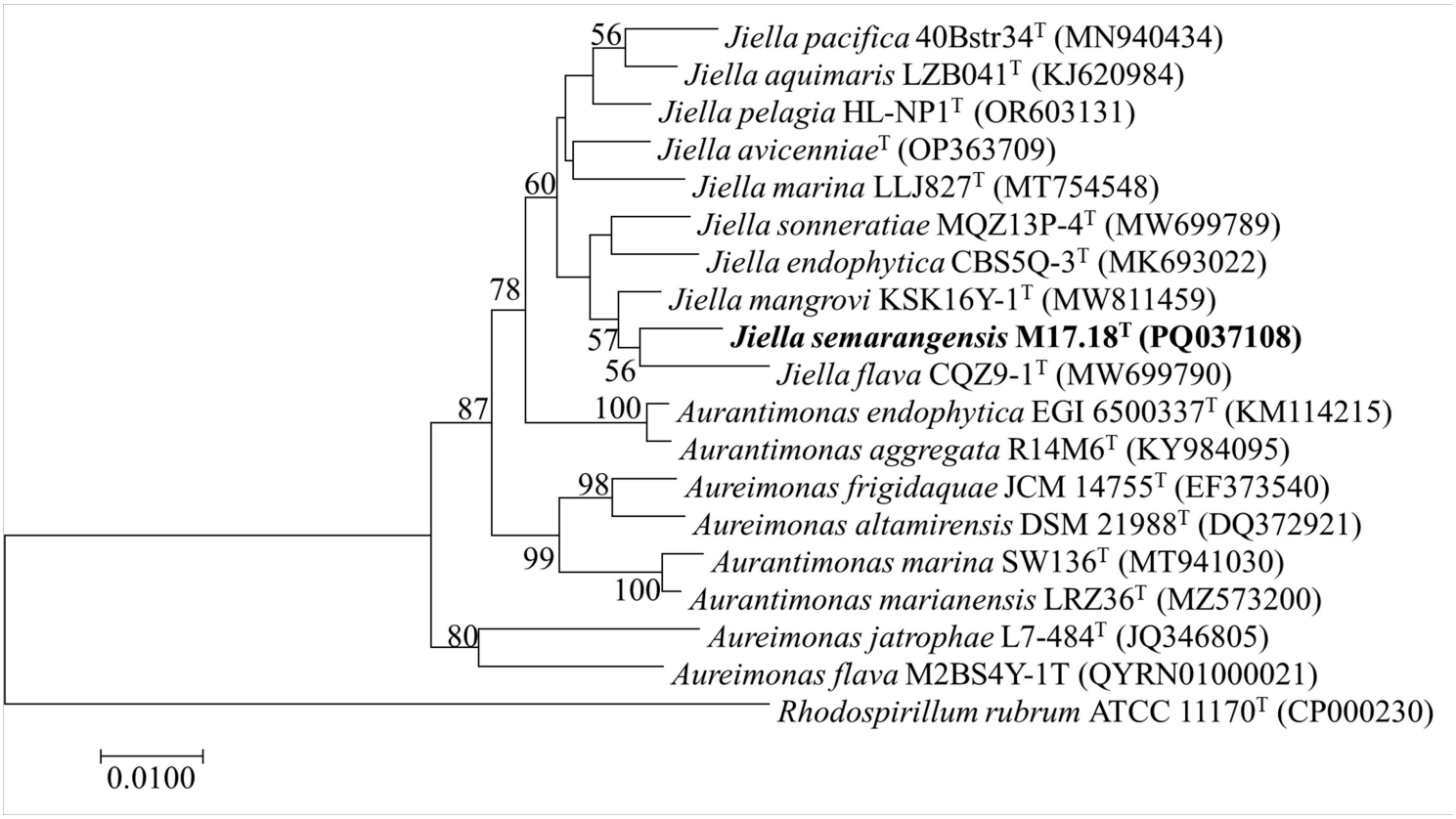


Figure 1

The reconstructed neighbour-joining tree showing the phylogenetic position of strain M17.18^T and the closely related species based on almost complete 16S rRNA gene sequences (1485 bp). *Rhodospirillum rubrum* ATCC 11170^T was used as an outgroup. Numbers at nodes refer to bootstrap values (based on 1000 replicates; only values > 50 % are shown). Bar, 0.01 substitutions per nucleotide position.

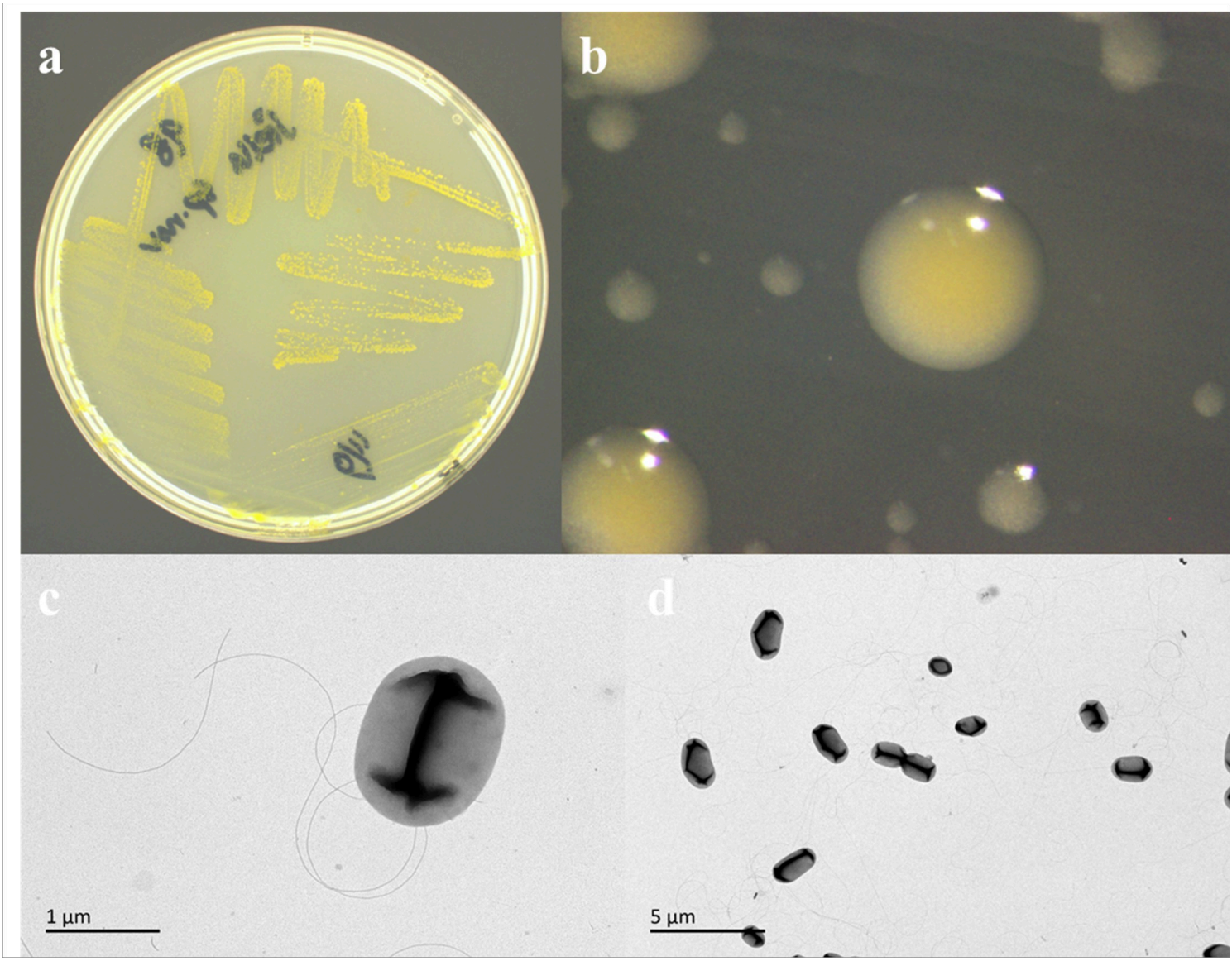


Figure 2

Morphological characteristics of strain M17.18^{T}, (a) plate colony, (b) single colony, (c-d) transmission electron microscopy (TEM) photographs

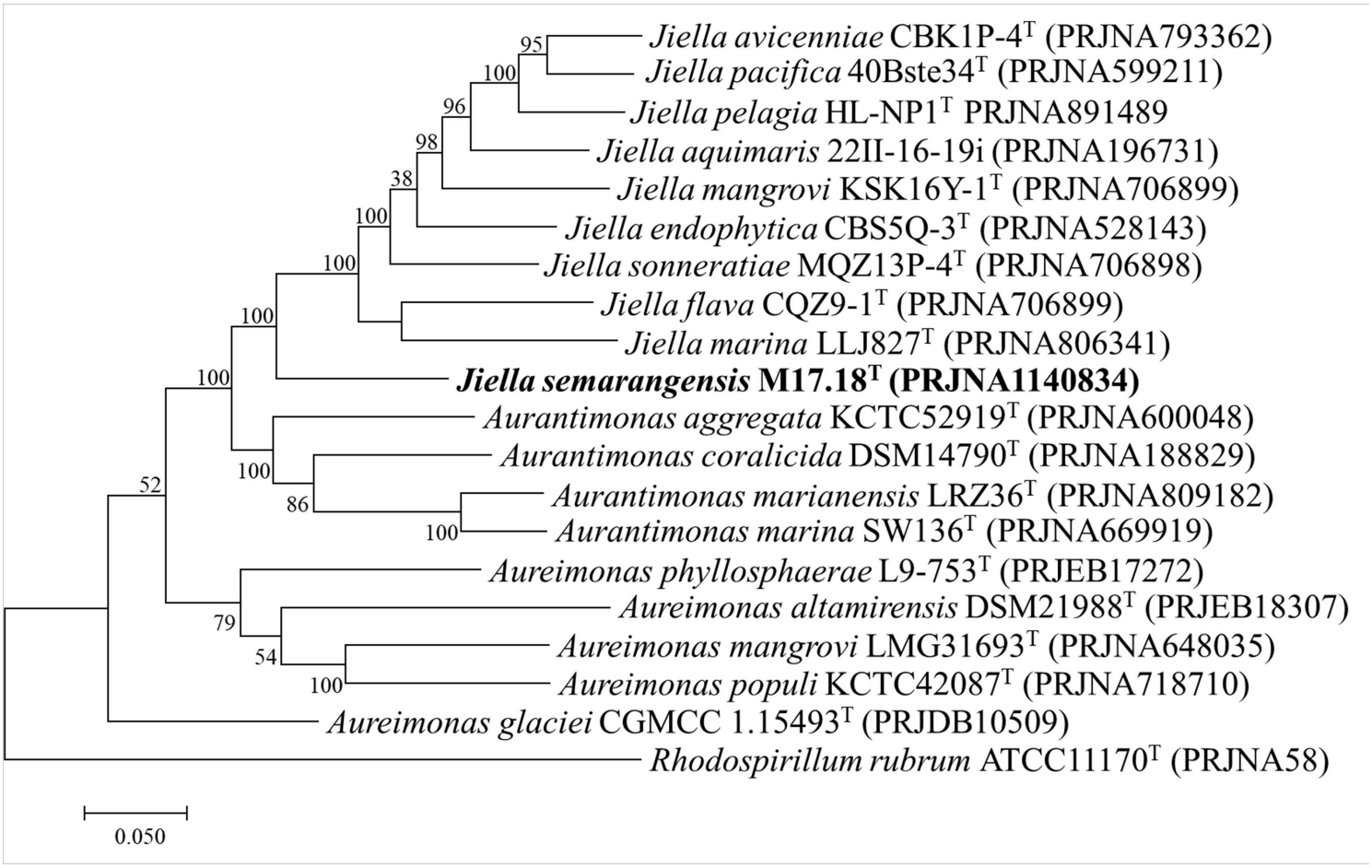


Figure 3

The maximum-likelihood phylogenetic tree constructed by autoMLST based on 86 concatenated core genes exhibiting the phylogenetic position of strain M17.18^T with the related species. *Rhodospirillum rubrum* ATCC 11170^T was used as the outgroup (based on 1000 replicates; only values > 50% are shown). Bar, 0.05 substitutions per nucleotide position.

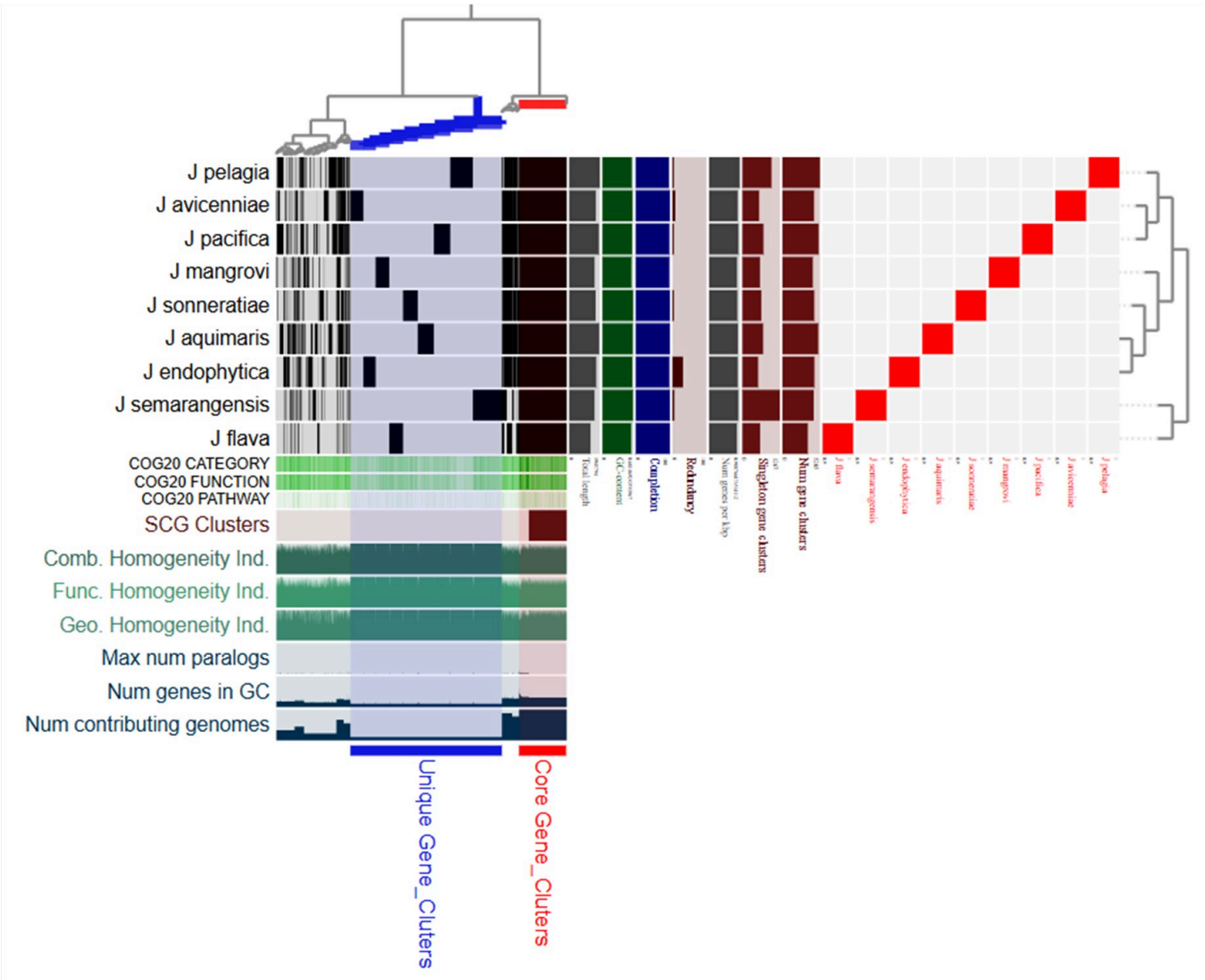


Figure 4

Pangenome analysis of the *Jiella* species using Anvi'o.

Supplementary Files

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