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# Proposal for reclassifying *Tellurirhabdus* species within the genus *Larkinella*

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## Abstract

**Background:** Members of the genera *Larkinella* and *Tellurirhabdus* are Gram-negative, aerobic, and consist of menaquinone (MK)-7 as their main isoprenoid quinone. Recent analysis suggests that both genera share similar characteristics. The taxonomic position of the genera *Larkinella* and *Tellurirhabdus* has been evaluated using genomic analysis.

**Methods:** The quality of the genomic sequences of *Larkinella* and *Tellurirhabdus* was assessed after they were downloaded from NCBI (<https://www.ncbi.nlm.nih.gov/>). Average nucleotide identity (ANI) and average amino acid identity (AAI) data was used for evaluating their genomic relatedness.

**Results:** The AAI values between *Larkinella* and *Tellurirhabdus* were above the threshold value of genus delineation (>60–65%) indicating that they are members of the same genus. The ANI values among *Larkinella* and *Tellurirhabdus* species were below 95–96% indicating they were different species.

**Conclusion:** We propose transferring *Tellurirhabdus bombi* to the genus *Larkinella* as *Larkinella bombi* comb. nov. and *Tellurirhabdus rosea* to the genus *Larkinella* as *Larkinella roseola* nom. nov. based on our research findings.



## Introduction

The genera *Larkinella* and *Tellurirhabdus* were proposed by Vancanneyt *et al.*, [1] and Choi *et al.*, [2] respectively. The genus *Larkinella* consists of 11 species with validly published names (<https://lpsn.dsmz.de/genus/larkinella>; accessed on 1/11/2024) [3], while the genus *Tellurirhabdus* consists of two species with validly published names (<https://lpsn.dsmz.de/genus/tellurirhabdus>; accessed on 1/11/2024) [3]. Members of the genera *Larkinella* and *Tellurirhabdus* exhibit similar characteristics, including being Gram-negative, aerobic, and having MK-7 as their main isoprenoid quinone [1, 2]. *Larkinella* species were reported from various ecological niches like water of a steam generator [1], soil [4, 5], manganese mine soil [6], fermented bovine products [7], decomposing wood [8], beach sand [9], etc. *Tellurirhabdus* species were reported from soil [2] and bumblebee [10]. Recently, during the description of one *Tellurirhabdus* novel species (*Tellurirhabdus bombi*) it was observed that *Tellurirhabdus* species shared similar features as that of *Larkinella* species [10] and hence the present study was carried out to evaluate the taxonomic position of *Tellurirhabdus* species.

## Methods

Molecular Evolutionary Genetics Analysis (MEGA) software (version 7.0) [11] was used to construct phylogenetic trees based on aligned 16S rRNA gene sequences (aligned using Clustal W [12]). The analyses utilized maximum-likelihood (ML) [13], neighbour-joining (NJ) [14], and maximum-parsimony (MP) [15] methods, each performed with 1000 bootstrap replications [16]. The evolutionary distances for the NJ and ML algorithms were calculated using Kimura's two-parameter model [17]. Uniform rates across sites were assumed, and alignment gaps or missing data at all sites were treated with complete deletion before tree reconstruction. For the ML tree, the initial trees for the heuristic search were generated automatically using the neighbour-joining and BioNJ algorithms, based on a pairwise distance matrix computed with the maximum composite likelihood method [18]. The genome sequence of *Tellurirhabdus* and *Larkinella* species namely *Tellurirhabdus bombi* IE-0392<sup>T</sup> (CP090557), *Tellurirhabdus rosea* U15<sup>T</sup> (CP111085), *Larkinella soli* MIMbqt9<sup>T</sup> (QTJY000000000), *Larkinella insperata* LMG 22510<sup>T</sup> (CP110973), *Larkinella arboricola* DSM 21851<sup>T</sup> (QLMC000000000), *Larkinella terrae* KCTC 52001<sup>T</sup> (WJXZ000000000), *Larkinella rosea* KCTC 52004<sup>T</sup> (RQJO000000000), *Larkinella punicea* ZZJ9<sup>T</sup> (QOWE000000000), and *Larkinella knui* KCTC 42998<sup>T</sup> (RQJP000000000) were downloaded from NCBI (<https://www.ncbi.nlm.nih.gov/>). *Dyadobacter fermentans* DSM 18053<sup>T</sup> (CP001619) used as an

outgroup was also downloaded from NCBI (<https://www.ncbi.nlm.nih.gov/>). The genome size and G+C content were calculated using an in-house Perl script, while genome completeness and contamination were assessed with CheckM [19]. The presence of tRNA was detected using tRNAscan-SE [20]. Type Strain Genome Server (TYGS) was used to construct the phylogenomic tree [21] and the result was visualized using Interactive Tree of Life (iTOL) version 6 [22]. The average amino acid identity value (AAI) was calculated using CompareM (<https://github.com/dparks1134/CompareM> [23, 24]). The average nucleotide identity (ANI) value was calculated using the Pyani software package, which included the ANIb parameter [25, 26].

## Results

The genome sizes of the *Tellurirhabdus* and *Larkinella* species ranged from 4941522 to 8175905 bp, with G+C content ranging from 48.8 to 57.6%. The number of tRNAs ranged from 39 to 50. The genome was >90% complete, with <5% contamination. Detailed genome traits of *Tellurirhabdus* and *Larkinella* species are mentioned in Table 1. In the present study, the ANI values (Table 2) among *Larkinella*, *Tellurirhabdus*, and *Dyadobacter* species were below 95-96%. In the phylogenomic tree, *Larkinella* and *Tellurirhabdus* species showed a distinct clade (Figure. 1). A similar clade was noticed when the trees were constructed using NJ, ML, and MP methods (Figure S1, S2, and S3). In the present study, the AAI values among *Larkinella* and *Tellurirhabdus* were evaluated. Based on the data in Table 3, the AAI value between *Tellurirhabdus rosea* U15<sup>T</sup> and the analyzed *Larkinella* species ranged from 68.7% to 70.1%, while the value between *Tellurirhabdus bombi* IE-0392<sup>T</sup> and the *Larkinella* species ranged from 70.8% to 71.3%.

Genomes attributes	Size (bp)	G+C content (%)	Completeness (%)	Contamination (%)	tRNAs
<i>Larkinella rosea</i> KCTC 52004 <sup>T</sup>	8175905	50.8	100	0.6	50
<i>Larkinella terrae</i> KCTC 52001 <sup>T</sup>	7847997	51.2	100	0.6	42
<i>Larkinella soli</i> MIMbqt9 <sup>T</sup>	6928969	57.6	100	0.3	45
<i>Tellurirhabdus rosea</i> U15 <sup>T</sup>	5582807	57.0	100	0.89	46
<i>Larkinella punicea</i> ZZJ9 <sup>T</sup>	8006526	51.7	100	1.49	41
<i>Larkinella knui</i> KCTC 42998 <sup>T</sup>	7652485	51.3	100	0.6	42
<i>Larkinella humicola</i> MA1 <sup>T</sup>	7857116	52.2	100	0.89	39
<i>Tellurirhabdus bombi</i> IE-0392 <sup>T</sup>	4941522	48.8	99.7	2.08	41
<i>Larkinella insperata</i> LMG 22510 <sup>T</sup>	6564943	53.2	99.7	0.3	41
<i>Larkinella arboricola</i> DSM 21851 <sup>T</sup>	7016227	51.8	99.7	0.6	39

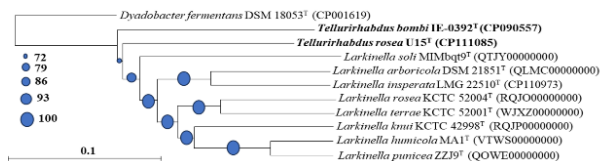
**Table 1:** Genomic traits of *Tellurirhabdus* and *Larkinella* species.

ANiB (%)	1	2	3	4	5	6	7	8	9	10	11
1)	-	71.1	71.0	71.2	71.0	71.1	70.9	71.0	71.1	70.5	71.2
2)	71.0	-	78.8	85.3	78.5	78.8	78.3	75.1	78.4	73.3	74.0
3)	71.1	78.7	-	78.4	82.3	91.9	80.3	75.1	80.4	73.1	73.8
4)	71.2	85.4	78.6	-	78.3	78.7	78.1	75.3	78.2	73.3	74.1
5)	70.8	78.5	82.4	78.1	-	82.6	80.3	74.8	80.3	72.9	73.5
6)	71.1	78.7	92.0	78.5	82.5	-	80.5	75.2	80.6	73.2	73.8
7)	70.9	78.1	80.3	77.9	80.2	80.5	-	74.5	85.3	73.0	73.4
8)	70.9	75.1	75.2	75.2	74.8	75.2	74.7	-	75.0	72.4	74.7
9)	71.0	78.3	80.5	78.1	80.2	80.6	85.4	74.9	-	72.9	73.7
10)	70.5	73.3	73.1	73.3	73.1	73.2	73.1	72.5	73.1	-	73.5
11)	71.4	74.0	73.9	74.1	73.7	74.1	73.6	74.9	73.8	73.5	-

**Table 2:** Average nucleotide identity (ANI) value among genera *Larkinella*, *Tellurirhabdus* and *Dyadobacter*. Note: 1) *Dyadobacter fermentans* DSM 18053<sup>T</sup>, 2) *Larkinella arboricola* DSM 21851<sup>T</sup>, 3) *Larkinella humicola* MA1<sup>T</sup>, 4) *Larkinella insperata* LMG 22510<sup>T</sup>, 5) *Larkinella knui* KCTC 42998<sup>T</sup>, 6) *Larkinella punicea* ZZJ9<sup>T</sup>, 7) *Larkinella rosea* KCTC 52004<sup>T</sup>, 8) *Larkinella soli* MIMbqt9<sup>T</sup>, 9) *Larkinella terrae* KCTC 52001<sup>T</sup>, 10) *Tellurirhabdus bombi* IE-0392<sup>T</sup>, 11) *Tellurirhabdus rosea* U15<sup>T</sup>.

AAI (%)	1	2	3	4	5	6	7	8	9	10	11
1)	-	71.0	94.8	59.9	81.5	83.8	81.7	83.7	86.4	74.1	69.1
2)	71.0	-	70.9	60.0	71.2	70.8	71.3	70.8	71.0	70.8	72.9
3)	94.8	70.9	-	60.2	81.4	83.8	81.7	83.9	86.4	74.3	69.1
4)	59.9	60.0	60.2	-	60.4	60.1	60.6	60.2	60.1	60.0	59.8
5)	81.5	71.2	81.4	60.4	-	80.8	90.2	80.8	81.2	74.5	69.4
6)	85.8	70.8	83.8	60.1	80.8	-	80.9	89.5	83.7	73.7	68.9
7)	81.7	71.3	81.7	60.6	90.2	80.9	-	80.9	81.5	74.7	69.7
8)	83.7	70.8	83.9	60.2	80.8	89.5	80.9	-	83.5	73.7	68.7
9)	86.4	71.0	86.4	60.1	81.2	83.7	81.5	83.5	-	74.0	69.1
10)	74.1	70.8	74.3	60.0	74.5	73.7	74.7	73.7	74.0	-	70.1
11)	69.1	72.9	69.1	59.8	69.4	68.9	69.7	68.7	69.1	70.1	-

**Table 3:** Average amino acid (AAI) identity value among genera *Larkinella*, *Tellurirhabdus* and *Dyadobacter*. Note: 1) *Larkinella punicea* ZZJ9<sup>T</sup>, 2) *Tellurirhabdus bombi* IE-0392<sup>T</sup>, 3) *Larkinella humicola* MA1<sup>T</sup>, 4) *Dyadobacter fermentans* DSM 18053<sup>T</sup>, 5) *Larkinella insperata* LMG 22510<sup>T</sup>, 6) *Larkinella rosea* KCTC 52004<sup>T</sup>, 7) *Larkinella arboricola* DSM 21851<sup>T</sup>, 8) *Larkinella terrae* KCTC 52001<sup>T</sup>, 9) *Larkinella knui* KCTC 42998<sup>T</sup>, 10) *Larkinella soli* MIMbqt9<sup>T</sup>, 11) *Tellurirhabdus rosea* U15<sup>T</sup>.



**Figure 1:** Phylogenomic tree showing the position of *Larkinella* and *Tellurirhabdus* species. *Dyadobacter fermentans* DSM 18053<sup>T</sup> was used as an out-group. Tree scale 0.1. Bootstrap support values (based on 1000 replicates) are indicated by the size of the blue circles at the nodes, with the key shown in the upper left

## Discussion

The genera *Larkinella* and *Tellurirhabdus* are the members of the family *Spirosomataceae* [3]. Both genera are Gram-negative and aerobic [1, 2]. Recently Zhang *et al.*, reported that *Tellurirhabdus* species share phenotypic and chemotaxonomic characteristics similar to *Larkinella* species [10]. The present study reviewed the taxonomic status of the taxa *Larkinella* and *Tellurirhabdus*. The genomes were downloaded and their quality assessed using CheckM, as it offers methods for assessing genome quality and providing reliable estimates of completeness and contamination by utilizing ubiquitous, single-copy genes within a phylogenetic lineage [19]. Good-quality genomes exhibit completeness levels more than 90% and contamination levels less than 5% [19]. The *Larkinella* and *Tellurirhabdus* genomes downloaded from NCBI have high completeness and low contamination

levels (>90 and <5%, respectively), indicating high quality genomes [19].

The ANI value was introduced for species-level delineation and a cut-off value of 95–96% was proposed [27]. In the present study, the ANI values among *Larkinella*, *Tellurirhabdus*, and *Dyadobacter* species were below 95–96% indicating they were different species [27]. Similarly, AAI was introduced to delineate strains at the genus level by measuring the average amino acid identity of all shared genes between two strains, allowing for an assessment of their genetic relatedness [28] and the threshold value of >60–65% was also proposed [28, 29]. The AAI values between *Larkinella* and *Tellurirhabdus* were above the threshold value of genus delineation (>60–65%) indicating that they were members of the same genus. Since the genus *Larkinella* was published earlier than *Tellurirhabdus* [1, 2] hence *Tellurirhabdus* species should be transferred to the genus *Larkinella*. We propose transferring *Tellurirhabdus bombi* as *Larkinella bombi* comb. nov. Since *Tellurirhabdus rosea* would be a later homonym of *Larkinella rosea*, a name already assigned to a species in the genus *Larkinella*, the name *Larkinella roseola* nom. nov. is proposed. In addition, the culture deposition certificates of *Tellurirhabdus* species are depicted in Figure S4.

The taxonomic position of the genera *Larkinella* and *Tellurirhabdus* was evaluated using genome analysis. The genome size of *Larkinella* and *Tellurirhabdus*

species ranged from 4941522-8175905 bp and their G+C content ranged from 48.8-57.6%. The number of tRNAs ranged from 39-50. CheckM analysis revealed that *Larkinella* and *Tellurirhabdus* species genome completeness and contamination were >90 and <5%, respectively indicating good-quality genomes. The ANI values among *Larkinella* and *Tellurirhabdus* species were below the cut-off for species delineation indicating they were different species. However, the AAI values were above the cut-off value (>60–65%) for genus delineation indicating they belong to the same genus. Based on the above results, we propose to transfer *Tellurirhabdus rosea* to the genus *Larkinella* as *Larkinella roseola* nom. nov., and *Tellurirhabdus bombi* as *Larkinella bombi* comb. nov.

*Larkinella roseola* (ro.se'o.la. N.L. fem. adj. *roseola*, rose-colored, pink).

Basonym: *Tellurirhabdus rosea* Choi *et al.*

The description of this species is the same as provided by Choi *et al.*, [2] for *Tellurirhabdus rosea*. The type strain is U15<sup>T</sup> (= KCTC 62116<sup>T</sup> = JCM 32361<sup>T</sup>). *Larkinella bombi* (bom'bi. L. n. *bombus* a boom, a deep hollow noise, buzzing, also the zoological genus name of the bumblebee; N.L. gen. n. *bombi* of *Bombus*, of a bumblebee).

Basonym: *Tellurirhabdus bombi* Zhang *et al.*

The description of this species is the same as provided by Zhang *et al.*, [10] for *Tellurirhabdus bombi*. The type strain is IE-0392<sup>T</sup> (= GDMCC 1.2794<sup>T</sup> = JCM 35040<sup>T</sup>).

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## Data Availability

All datasets generated or analysed during this study are included in the manuscript.

## Ethics Statement

This article does not contain any studies on human participants or animals performed by the authors.

## Author Contributions

Syed Raziuddin Quadri conceptualized, supervised, and edited the article. Wesam Nofal and Muhannad

Alruwaili performed the literature search and conducted the genome analysis.

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