

Partial 18S ribosomal DNA sequences of nematode species collected in South Korea

Jihye Yoon^{1*}, Daye Kwon^{2*}, Jun Kim^{3§}, Junho Lee^{4§}

¹. Institute of Molecular Biology and Genetics, Seoul National University, Seoul 08826, Korea

². Department of Biological Sciences, Seoul National University, Seoul 08826, Korea

³. Research Institute of Basic Sciences, Seoul National University, Seoul 08826, Korea

⁴IMBG, RIBS, Department of Biological Sciences, Seoul National University, Seoul 08826, Korea

[§]To whom correspondence should be addressed: dauer@snu.ac.kr; elegans@snu.ac.kr

*These authors contributed equally.

Abstract

Free-living nematodes are important model organisms in biology, and they can be collected from various materials including rotten fruit, plant, and soil. In order to explore the diversity of free-living nematodes in South Korea, we collected and isolated nematodes from rotten fruit matter from Seoul and Jeju island. Here, we report partial 18S ribosomal DNA sequences of the nematodes that we collected in South Korea between June and July of 2021. Three newly identified sequences are included.

A OM688231.1 to DQ285636.1

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Query 1   ACTGCATTGAATTGTTGTGCGGTT-AAAAGCTCGTAGTTGGATCTGTACATGATAGGTT 59
          |
Sbjct 577 ACTGCATTGAATTGTTGTGCGGTTAAAAGCTCGTAGTTGGATCTGTACATGATAGGTT 636
          |
Query 60   AGTTCATCCTTTGGATTGTAACATACTAAATGTTTTTATAAGATTTATATATTTTT 119
          |
Sbjct 637   TGTTCATCCTTTGGATTGTAACATACTAAATGTTTTTATAAGATTTTATAAGATTTT 692
          |
Query 120  tcttcgggggattatataatgctctatgaatttacttGAATAAATCAGAGTGCCTCAA 179
          |
Sbjct 693   TCTTT--ACGGA-GATATA-ATGTCTTATGAGTTTACTTTGAATAAATCAGAGTGCCTCAA 748
          |
Query 180  AACAGGCGTAAGCTTGTATGGTTTTGCATGGAATAATAGAACATGACTATGGTTCATGTT 239
          |
Sbjct 749  AACAGGCGTAAGCTTGTATGGTTTTGCATGGAATAATAGAACATGACTATGGTTCATGTT 808
          |
Query 240  TT-ATTGGTTTTTACTTACCATAGTAATGATTAAGGAACAACCGGGGATCCGATCG 298
          |
Sbjct 809  TTCAATGGTTTTTACTTACCATAGTAATGATTAAGGAACAACCGGGGATCCGATCG 868
          |
Query 299  CAGGGCGAGAGGTGAAATTCGTGGACCTTGCAGAGCAACTACAGCGAAGGCATTGGCC 358
          |
Sbjct 869  CAGGGCGAGAGGTGAAATTCGTGGACCTTGCAGAGCAACTACAGCGAAGGCATTGGCC 928
          |
Query 359  AAGAAATGTTTTCAATTAATCAAGAACGAAAGTCAGAGGATCGAAGGCGATCAGATCCGCC 418
          |
Sbjct 929  AAGAAATGTTTTCAATTAATCAAGAACGAAAGTCAGAGGATCGAAGGCGATCAGATCCGCC 988
          |
Query 419  CTAGTCTGACCGTAAACTATGCCATCTAGCGAGTTTCGGGTGGATATTTTATATTGCC 478
          |
Sbjct 989  CTAGTCTGACCGTAAACTATGCCATCTAGCGAGTTTCGGGTGGATATTTTATATTGCC 1048
          |
Query 479  CTTGAAACGAGCTTTCCGGAAACGAAAGTCTTTCGGTTCGGGGGGAAGTATGGTGCAAA 538
          |
Sbjct 1049 CTTGAAACGAGCTTTCCGGAAACGAAAGTCTTTCGGTTCGGGGGGAAGTATGGTGCAAA 1108
          |
Query 539  GTTGAACCTAAAGGAATTGACGGAAGGTCACCAACGAAAGTGAAGCCTCGCG 591
          |
Sbjct 1109 GTTGAACCTAAAGGAATTGACGGAAGGTCACCAACGAAAGTGAAGCCTCGCG 1161
    
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B OM688232.1 to EU040129.1

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Query 1   TTGAATTGTTGTGCGGTTAAAAGCTCGTAGTTGGATCTGTACATGATAGGTTAGTTCA 60
          |
Sbjct 169  TTGAATTGTTGTGCGGTTAAAAGCTCGTAGTTGGATCTGTACATGATAGGTTAGTTCA 228
          |
Query 61   TCCTTTGGATTGTAACATACTAAATGTTTTTATTAGATTTTTATATTTCTTTACGGA 120
          |
Sbjct 229  TCCTTTGGATTGCACTAACTAAATGTTTTTATTAGATTTTTATATTTCTTTACGGA 288
          |
Query 121  AATAAATGTCATATGAGTTTACTTTGAATAAATCAGAGTCTCAAAACAGGCGTAAGCT 180
          |
Sbjct 289  AATAAATGTCATATGAGTTTACTTTGAATAAATCAGAGTCTCAAAACAGGCGTAAGCT 348
          |
Query 181  TGTATGGTTTTGCATGGAATAATAGAACATGACTATGGTTCATGTTTTTATGGTTTTACT 240
          |
Sbjct 349  TGTATGGTTTTGCATGGAATAATAGAACATGACTATGGTTCATGTTTTTATGGTTTTACT 408
          |
Query 241  TACCATAGTAATGATTAAGGAACAACCGGGGATCCGATCGCAGGGCGAGAGGTGA 300
          |
Sbjct 409  TACCATAGTAATGATTAAGGAACAACCGGGGATCCGATCGCAGGGCGAGAGGTGA 468
          |
Query 301  AATTGCGGACCTTGCAGAGCAACTACAGCGAAGGCATTGGCAAGAATGTTTTCAATT 360
          |
Sbjct 469  AATTGCGGACCTTGCAGAGCAACTACAGCGAAGGCATTGGCAAGAATGTTTTCAATT 528
          |
Query 361  AATCAAGAACGAAAGTCAGAGGATCGAAGGCGATCAGATACCGCCCTAGTTCTGACCSTA 420
          |
Sbjct 529  AATCAAGAACGAAAGTCAGAGGATCGAAGGCGATCAGATACCGCCCTAGTTCTGACCSTA 588
          |
Query 421  AACTATGCCATCTAGCGAGTTTCGGGTGGATATTTATATTGCCCTTGAACGAGCTTC 480
          |
Sbjct 589  AACTATGCCATCTAGCGAGTTTCGGGTGGATATTTATATTGCCCTTGAACGAGCTTC 648
          |
Query 481  CCGAAACGAAAGCTTTCGGTTCGGGGGGAAGTATGGTGCAAAGTTGAAACCTAAAGG 540
          |
Sbjct 649  CCGAAACGAAAGCTTTCGGTTCGGGGGGAAGTATGGTGCAAAGTTGAAACCTAAAGG 708
          |
Query 541  AATTGACGGAAGGTCACCAACGAAAGTGGAGCCTCGCG 578
          |
Sbjct 709  AATTGACGGAAGGTCACCAACGAAAGTGGAGCCTCGCG 746
    
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C OM688233.1 to MT548590.1

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Query 130  TTACTGTTG-ATAAATTGAAGTGTCAAGGCAAGCGATTGCTTGAACGTTTAACTATGGA 188
          |
Sbjct 637  TTACT-TTGAATAAAACAGAGTGTCAAAACAGCGATTGTTGAATGCTCGATCATGGA 695
          |
Query 189  ATAAAGGAGGAGAACTCTC-ATTGTTGGTTTTGTAGAGTTT-ATAAGATTAAAG 244
          |
Sbjct 696  ATAAAGGAAATAGGATCTAGGTTCTATTATTGG--TTCAAGAAATTAGATAATGATTAAG 753
          |
Query 245  AGGGAATAATTCGGGGTACTCGTATCTTTAGGCGAGGGGTGAAATCGTGAACCTAGAG 304
          |
Sbjct 754  AGAGACAATTCGGGGGATTCGTATCCCTACGCGAGAGGTGAAATTCGTGAACCTAGAG 813
          |
Query 305  AGACGATCAAAAGCGAAAGCAGGTACCAAGAAATCTCTTATTAAATCAAGAACGAAAGTCA 364
          |
Sbjct 814  GGACGCCCAAAAGCGAAAGCATTGGCAAGAAATGCTCTCAATTAATCAAGAACGAAAGTCA 873
          |
Query 365  GAGGATCGAAGGTGATTAGATACCGCCCTAGTTCTGACGCGTAAACGATACCATCTAACGA 424
          |
Sbjct 874  GAGGTTGGAAGGCGATTAGATACCGCCCTAGTTCTGACGCGTAAACTATGCGATCTAGCGA 933
          |
Query 425  TCACTTGGAGCTTTTGT-ACCTCAAATGGGAGTTTCCCGGAAACGAAAGTTTTTCGGTTC 483
          |
Sbjct 934  TCCGATGG-GTTTTTTTGACTT-GTCGGGGAGCTTCCCGGAAACGAAAGCTTTTCGGTTC 991
          |
Query 484  TGGGGGTAGTATGGTTGCAAGCTGAACTTAAAGAAATTTGACGGAAGGGCACCACAAAG 543
          |
Sbjct 992  CGGGGGTAGTATGGTTGCAAGCTGAACTTAAAGAAATTTGACGGAAGGGCACCACAAAG 1051
          |
Query 544  AGTGAGC 551
          |
Sbjct 1052 AGTGAGC 1059
    
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Figure 1. Pairwise alignments between rDNA sequences of the three previously reported species and the new nematode sequences identified in this study.

Red arrowheads represent gaps and blue arrowheads represent mismatches. (A) Alignment of our OM688231.1 sequence to rDNA sequence of *Panagrolaimus cf. rigidus* (DQ285636.1). (B) Alignment of our OM688232.1 sequence to rDNA sequence of *Panagrolaimus* sp. FL-SType-9 (EU040129.1). (C) Alignment of our OM688233.1 sequence to rDNA sequence of *Oscheius chongmingensis* (MT548590.1).

Description

Free-living nematodes are important model organisms in biology, and they can be collected from various materials including rotten fruit, plant, and soil (Barrière and Félix 2005; Nigon and Félix 2017). In order to explore the diversity of free-living nematodes in South Korea, we collected and isolated nematodes from rotten fruit matter from Seoul and Jeju island. Then, we cultured them and extracted DNA to identify species through partial ribosomal RNA gene (rDNA) sequencing. Here, we report a subset of species that we collected in South Korea during June and July of 2021 (Table 1).

Among 30 different samples collected from 14 rotten fruits, *Caenorhabditis briggsae* was the major species (n=15), followed by *Chylorhabditis epuraeae* (n=5). While most of the fruits had been occupied by one dominant species, we observed multiple species from 2 out of the 14 fruits (Samples 1 and 9 in Table 1). In addition, the majority (n=24) of the samples matched with the preexisting species and their rDNA sequences on the NCBI database, including *Auanema freiburgensis* (KY680647.1) (Isolate 1), *Caenorhabditis briggsae* (MN519141.1) (Isolates 2–16), *Chylorhabditis epuraeae* (LC570777.1) (Isolates 17–21), *Oscheius* sp. IR (MW667590.1) (Isolate 24), and *Oscheius tipulae* (MH983026.1) (Isolates 25–26).

Interestingly, we identified three species (n=6) that do not have perfect matching preexisting rDNA sequences on the NCBI database (Figure 1). Initially, these samples were determined to be closest to *Panagrolaimus cf. rigidus* (DQ285636.1; n=2; Isolates 27–28), *Panagrolaimus* sp. FL-SType-9 (EU040129.1; n=2; Isolates 29–30) and *Oscheius chongmingensis* (MT548590.1; n=2; Isolates 22–23). However, each of their identity matched with *Panagrolaimus cf. rigidus* by 98% (579/592; Figure 1A), *Panagrolaimus* sp. FL-SType-9 by 99% (575/578; Figure 1B), and *Oscheius chongmingensis* by 83% (355/428; Figure 1C). The two isolates obtained from each species had identical variants and such variants were confirmed manually by examining graphical variant peaks. Thus, we concluded that the mismatch in identity in each species could be seen as real variants and not a sequencing error. This suggests that they are either novel species, species without reported rDNA sequences, or genetically divergent individuals of previously reported species. Therefore, in this paper, we report three rDNA sequences that have not been reported earlier. Their partial rDNA sequences are available in the NCBI database under accession numbers OM688231–OM688233.

Table 1. Sampling information

Isolate	Sample	Date	(Latitude, longitude)	Material	Location	Closest species name	Sequence ID	Accession of newly identified sequences
1	1	7/9/2021	(33.26189,126.42172)	Tangerine	Jeju island	<i>Auanema freiburgensis</i>	KY680647.1	
2	2	6/29/2021	(37.45424,126.95374)	Persimmon	Seoul	<i>Caenorhabditis briggsae</i>	MN519141.1	
3	2	6/29/2021	(37.45424,126.95374)	Persimmon	Seoul	<i>Caenorhabditis briggsae</i>	MN519141.1	
4	2	6/29/2021	(37.45424,126.95374)	Persimmon	Seoul	<i>Caenorhabditis briggsae</i>	MN519141.1	

5	2	6/29/2021	(37.45424,126.95374)	Persimmon	Seoul	<i>Caenorhabditis briggsae</i>	MN519141.1	
6	3	6/29/2021	(37.45424,126.95374)	Persimmon	Seoul	<i>Caenorhabditis briggsae</i>	MN519141.1	
7	3	6/29/2021	(37.45424,126.95374)	Persimmon	Seoul	<i>Caenorhabditis briggsae</i>	MN519141.1	
8	3	6/29/2021	(37.45424,126.95374)	Persimmon	Seoul	<i>Caenorhabditis briggsae</i>	MN519141.1	
9	4	7/2/2021	(37.56210,126.89571)	Armenian plum	Seoul	<i>Caenorhabditis briggsae</i>	MN519141.1	
10	4	7/2/2021	(37.56210,126.89571)	Armenian plum	Seoul	<i>Caenorhabditis briggsae</i>	MN519141.1	
11	4	7/2/2021	(37.56210,126.89571)	Armenian plum	Seoul	<i>Caenorhabditis briggsae</i>	MN519141.1	
12	1	7/9/2021	(33.26189,126.42172)	Tangerine	Jeju island	<i>Caenorhabditis briggsae</i>	MN519141.1	
13	5	7/9/2021	(33.26191,126.42175)	Tangerine	Jeju island	<i>Caenorhabditis briggsae</i>	MN519141.1	
14	5	7/9/2021	(33.26191,126.42175)	Tangerine	Jeju island	<i>Caenorhabditis briggsae</i>	MN519141.1	
15	5	7/9/2021	(33.26191,126.42175)	Tangerine	Jeju island	<i>Caenorhabditis briggsae</i>	MN519141.1	
16	6	7/9/2021	(33.26183,126.42200)	Tangerine	Jeju island	<i>Caenorhabditis briggsae</i>	MN519141.1	
17	7	7/23/2021	(37.55013,127.16773)	Chinese plum	Seoul	<i>Chylorhabditis epuraeae</i>	LC570777.1	
18	7	7/23/2021	(37.55013,127.16773)	Chinese plum	Seoul	<i>Chylorhabditis epuraeae</i>	LC570777.1	
19	7	7/23/2021	(37.55013,127.16773)	Chinese plum	Seoul	<i>Chylorhabditis epuraeae</i>	LC570777.1	
20	8	7/19/2021	(37.56202,126.89667)	Persimmon	Seoul	<i>Chylorhabditis epuraeae</i>	LC570777.1	
21	8	7/19/2021	(37.56202,126.89667)	Persimmon	Seoul	<i>Chylorhabditis epuraeae</i>	LC570777.1	

22	1	7/9/2021	(33.26189,126.42172)	Tangerine	Jeju island	<i>Oscheius chongmingensis</i>	MT548590.1	OM688233
23	9	7/9/2021	(33.26192,126.42178)	Tangerine	Jeju island	<i>Oscheius chongmingensis</i>	MT548590.1	OM688233
24	10	7/9/2021	(33.26193,126.42194)	Tangerine	Jeju island	<i>Oscheius</i> sp. IR	MW667590.1	
25	9	7/9/2021	(33.26192,126.42178)	Tangerine	Jeju island	<i>Oscheius tipulae</i>	MH983026.1	
26	11	7/9/2021	(33.26187,126.42205)	Tangerine	Jeju island	<i>Oscheius tipulae</i>	MH983026.1	
27	12	6/9/2021	(33.26186,126.42227)	Tangerine	Jeju island	<i>Panagrolaimus</i> cf. <i>rigidus</i>	DQ285636.1	OM688231
28	13	6/9/2021	(33.26143,126.42175)	Tangerine	Jeju island	<i>Panagrolaimus</i> cf. <i>rigidus</i>	DQ285636.1	OM688231
29	14	6/9/2021	(33.26197,126.42221)	Tangerine	Jeju island	<i>Panagrolaimus</i> sp. FL-SType-9	EU040129.1	OM688232
30	14	6/9/2021	(33.26197,126.42221)	Tangerine	Jeju island	<i>Panagrolaimus</i> sp. FL-SType-9	EU040129.1	OM688232

Methods

For the nematode collection, we first collected rotten fruit matter - tangerine, persimmon, Armenian plum, Chinese plum - from Seoul and Jeju island. We isolated the nematodes on the day of collection. The fruits were washed and mixed with distilled water. After centrifugation, about 90% of the supernatant was removed and the pellet was mixed with the remaining supernatant. Then, the solution containing nematodes from each fruit was pipetted onto an NGM plate, and the nematodes were isolated to a new plate after 10 to 15 minutes for further growth. Once initial nematodes laid eggs, we singled-out 3 adult female or hermaphrodite individuals from each plate onto new NGM plates.

After the populated growth of singled-out samples, we performed partial rDNA sequencing with *nem1* and *nem2* as primers (Foucher and Wilson 2002). Up to 20 bp at the beginning and the end of the sequence were trimmed by hand to remove ambiguities and primer sequences. The sequence was also trimmed for low quality. Finally, for the species diagnosis, we searched for matching sequences and species using NCBI BLAST (NCBI Resource Coordinators 2018).

Reagents

Primer set (5' to 3')

nem1 (forward): GCA AGT CTG GTG CCA GCA GC

nem2 (reverse): CCG TGT TGA GTC AAA TTA AG

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References

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