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BIAS DISTRIBUTION OF *PRISTIONCHUS* NEMATODES FOUND IN CAT TIEN AND CUC PHUONG NATIONAL PARKS, VIETNAM

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SUMMARY

The *Pristionchus* nematodes are distributed in many places worldwide, so they should be diverse in many aspects of biology. The most updated species, *P. pacificus*, has many differences from and similarities to the standard model organism, *Caenorhabditis elegans*, and become a parallel but complementary laboratory model for research in genetics, diversity, and evolution. It therefore acquires the demonstration of wild-type isolates of the *Pristionchus* species in various habitats. In the survey of the soil nematodes, we collected vegetation samples from forests and used them to isolate nematodes. The nematodes were raised in the laboratory to determine their species. In this research, we report the finding of numerous strains within the genus *Pristionchus*. By comparing the 18S rDNA sequences as an advanced barcode for the classification of free-living nematodes, we identified them in two different species (*P. pacificus* and *P. chinensis*) and predicted one new *Pristionchus* species. Of them, *P. pacificus* strains are dominant, more in the north and less in the south of Vietnam, and they likely evolved independently over time. The *P. chinensis* and new *Pristionchus* sp. were less distributed and probably found by chance. This result extends the global diversity of the nematode genus *Pristionchus*, facilitating future studies of evolutionary nematodes.

Keywords: Beetles, *Caenorhabditis* nematodes, culture, isolation, *Pristionchus*

INTRODUCTION

Many soil nematodes are known as models for biological research concerning biological principles and application. Regularly, *Caenorhabditis* species are mainly used to investigate functional genomics and genetics with respect to behaviors and morphology. In contrast, the *Pristionchus* nematodes are in part a complementary, however, different model.

Pristionchus pacificus develops well in the laboratory, its anatomy was demonstrated and the genome sequence was annotated, providing lots of advantages for molecular research (Dieterich *et al.*, 2008; Rodelsperger *et al.*, 2014; Schroeder, 2021), for example, diversity within the species (Hong, Sommer, 2006; Rodelsperger *et al.*, 2014; Schroeder, 2021). It is a dioecious species with many microscopic differences from and similarities to *C. elegans*, and so is emerging as a parallel system to *C. elegans* in multiple aspects of biological concepts (i.e. evolution, genomics, and genetics) (Kiontke, Fitch, 2005; Rudel *et al.*, 2005; Hong, Sommer, 2006; Sommer, 2006; Rae *et al.*, 2012; Sinha *et al.*, 2012; Pires-daSilva, 2013; Sommer, McGaughran, 2013; Lightfoot *et al.*, 2016; Moreno *et al.*, 2016).

However, ecologically, in contrast to a few *C. elegans* found in Eastern Europe, *P. pacificus* possesses many wild-type variants worldwide. They are diverse in many constraints, for example, mouth morphology interaction with habitats (Werner *et al.*, 2017), pheromone sensing ability of beetles (Hong *et al.*, 2008), and other insects (Hong, Sommer, 2006). There are also multiple differences in biology between the *Caenorhabditis* and *Pristionchus* species. For example, *P. pacificus* entirely develops through three juvenal stages while the *Caenorhabditis* species have four stages (Felix *et al.*, 1999). The hierarchy structures of neuronal dendrites and synaptic connections were found to determine the behavior of *P. pacificus*, which is, at some levels, distinct from *C. elegans* (Hong *et al.*, 2019). These two genera directly interact with each other, for example, *C. elegans* is a prey of *P. pacificus* (Quach, Chalasani, 2020). The genome structure in *P. pacificus* is subject to predict the survival rates of individuals (Dieterich *et al.*, 2008). Taking together, the isolation of *Pristionchus* nematodes as a model organism acquires benefits for various aspects of research.

Pristionchus pacificus isolates were found in many places worldwide but none of them were ever reported in Vietnam. In the effort to investigate any close nematodes to the *Caenorhabditis* nematodes in the wild, we isolated, raised, and described numerous isolates of *P. pacificus* from Cat Tien and Cuc Phuong National Parks, Vietnam. These results confer an understanding of the ecology and the diversity of the species and they also facilitate the overall picture of the soil free-living nematodes.

MATERIALS AND METHODS

Media preparation

New Cheap Media No.18 (NcM18) was a mixture of 0.4 g of pig fat, 20 ml of mushroom solutions, 17 g of agar, 4 ml of 0.75g/L NaCl, and 1 L distilled water (Le *et al.*, 2021). Nematode Growth Media (NGM) was a mixture of 1 mL of 5 mg/mL cholesterol, 2.5 g of peptone, 1 mL of 1M $CaCl_2$, 1 mL of 1M $MgSO_4$, 25 mL 1M KPO_4, 17 g of agar, 4 mL of 0.75 g/mL NaCl, and 1 L water.

Nematode selection

One hundred vegetation samples were collected from different sites in Cuc Phuong, and Cat Tien National Parks.

Caenorhabditis strains were isolated as in our previous description (Le *et al.*, 2021). An amount of 5 to 10 g of each vegetation compost (leaves, fruits, and flowers) was put on the surface of one *Escherichia coli* OP50-seeded media petri plate (10 cm) and then incubated at room temperature (approximately 25 ^{o}C) for three days. Subsequently, each of the two worms (mode) per sample plate was transferred onto and grown on an OP50-seeded media petri plate (5 cm) in a (19 ± 1) ^{o}C incubator for several generations. Each worm developed its population and was defined as a strain.

Morphologically, the population of each strain on the plate was observed under a microscope (4X) for morphology. The appearance should be similar to *Caenorhabditis* species in the translucence, length, and shape of the body. Regarding sex, the strain that had a majority of females or hermaphrodites was the candidate of soil free-living nematodes.

Species determination

The total DNA solution of every candidate strain was prepared with the "Single Worm Lysis" protocol (Ahringer, 2006; Le *et al.*, 2023). Next, the 18S rDNA sequence was amplified using primers for the nematodes: SSU26R (5'-CATTCTTGGCAAATGCTTTCG-3') and (SSU18A (5'-AAAGATTAAGCCATGCATG-3') (Barriere, Felix, 2006)). After, the 18S rDNA solution in the PCR product mixture was washed off the remaining DNA using the Intron DNA Purification Kit. The 18S rDNA was sequenced by sequencing services using either one of the PCR primers. The sequence of each 18S rDNA was compared with the DNA database of the National Center for Biotechnology Information (NCBI) using the online BLASTnt software by NCBI (Le *et al.*, 2021). In the pop-up results, the first species match determined the species identification.

The 18S rDNA sequences were filed together and then submitted to GenBank on NCBI. GenBank only verified the submitted sequences that matched over 90% of the total length with the database during the BLAST similarity search analysis of the submission process. So, the sequences shorter than 90% of the total length were ruled out of this research.

Phylogenetic analysis

The qualified 18S rDNA sequences of the strains were aligned together and run for phylogeny. The phylogenetic tree was reconstructed using the Neighbor-Joining method in the MEGA11 program with 100 bootstraps (Tamura *et al.*, 2021).

RESULTS

We isolated, raised, and identified different *Pristionchu*s species from 80 different sites, each 40 from one park (Figure 1 and Table 1). Thirty-five wild-type strains have 99.20% to 100% identity to *P. pacificus* found in two sites, Taiwan (KX113519.1 of strain RS5811) (Herrmann *et al.*, 2016) and California, the United States of America (AF083010.1 of strain PS312) (Sommer, 2006), suggesting that they are *P. pacificus*. In contrast, one strain (CFB76) has a high identity to *P. chinensis* (MW017217.1 of strain RS6023) found in Yunnan Province, China, suggesting that this is *P. chinensis*. One strain (CFB53) has a remarkably low identity (84.10%) to the first hit, which is *P. pacificus* (KY914568.1 of strain SMS4) found in Kerman Province, Iran, suggesting this may be a new *Pristionchus* species. Moreover, many point mutations accumulated in the approximately 900-nt fragments of the 18S rDNA sequences between the paired strains (Table S1), partly causing complications during the intra-strain phylogenetic analysis.

The number of strains from Cuc Phuong National Park nearly doubles that from Cat Tien National Park, indicating that the diversity is higher in Cuc Phuong and lower in Cat Tien (Table 1). In the phylogenetic analysis, we revealed likely discrimination between the strains from two parks (Figure 2), giving a consistent bias pattern of the nematode strains with our previous reports of *Caenorhabditis* nematodes (Le, Nguyen, 2021; Le *et al.*, 2023). The strains from Cuc Phuong show 14 times of branch joins (from CFB76 to CFB15), while those from Cat Tien have 12 times (CFB32 to CFB45), indicating that the strains in Cuc Phuong might evolve independently and quite faster than those in Cat Tien.

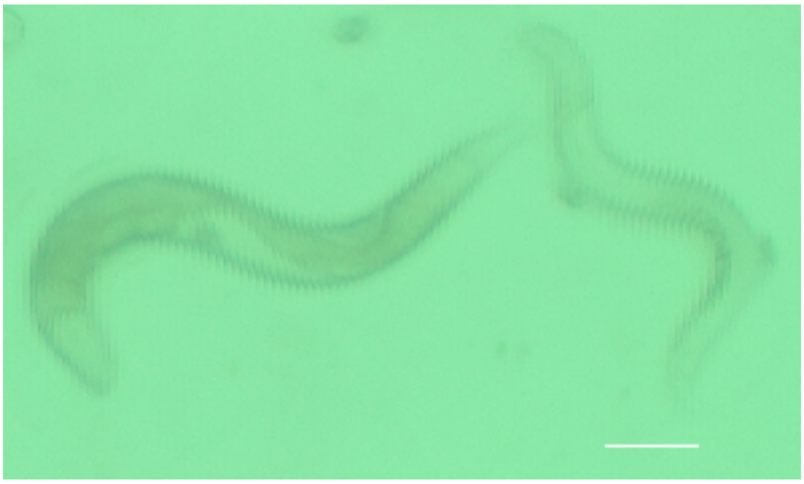

Figure 1. *Pristionchus pacificus* nematode. Two alive adult hermaphrodites are moving on an agar glass petri. Scale bar-100 µm.

Table 1. Molecular identification of *Pristionchus* strains.

No.	Strain	Species	Identity (%)/ Reference sequence	GenBank accession No.	Sample sites†
1	CFB137	*P. pacificus*	99.65/ KX113519.1	OR921133	cP29
2	CFB08	-	99.77/ -	OR921143	cP03
3	CFB31	-	99.77/ -	OR921144	cP36
4	CFB26	-	99.65/ -	OR921145	cP01
5	CFB14	-	99.77/ -	OR921146	cP33
6	CFB29	-	100.0/ -	OR921147	cP05
7	CFB22	-	99.53/ -	OR921151	-
8	CFB21	-	99.65/ -	OR921153	-
9	CFB17	-	99.42/ -	OR921148	cP24
10	CFB10	-	99.77/ -	OR921149	cP18
11	CFB15	-	99.88/ -	OR921150	cP20

12	CFB12	-	99.77/ -	OR921152	cP10
13	CFB28	-	99.53/ -	OR921154	cP04
14	CFB04	-	99.65/ -	OR921155	cP07
15	CFB53	*Pristionchus* sp.	84.10/ KY914568.1	OR921163	cT24
16	CFB24	*P. pacificus*	99.20/ AF083010.1	OR921134	cP03
17	CFB03	-	99.54/ -	OR921135	cP12
18	CFB11	-	99.77/ -	OR921136	cP20
19	CFB01	-	99.54/ -	OR921137	cP07
20	CFB19	-	99.77/ -	OR921138	cP09
21	CFB05	-	99.54/ -	OR921139	cP02
22	CFB18	-	99.88/ -	OR921140	cP31
23	CFB30	-	99.88/ -	OR921141	cP07
24	CFB27	-	99.42/ -	OR921166	cP04
25	CFB52	-	99.88/ -	OR921156	cT36
26	CFB39	-	99.42/ -	OR921157	cT37
27	CFB48	-	100.0/ -	OR921158	-
28	CFB46	-	99.54/ -	OR921159	cT08
29	CFB45	-	99.88/ -	OR921160	cT31
30	CFB42	-	99.65/ -	OR921161	cT35
31	CFB47	-	99.76/ -	OR921162	cT30
32	CFB32	-	99.53/ -	OR921164	cT25
33	CFB37	-	99.65/ -	OR921165	cT05
34	CFB36	-	99.88/ -	OR921167	cT22
35	CFB33	-	99.76/ -	OR921168	cT08
36	CFB35	-	99.42/ -	OR921169	cT12
37	CFB76	*P. chinensis*	99.01/ MW017217.1	OR921142	cP33

The comparison and submission were operated on December 9, 2023. Dash (-): identical to the upper notation. † cP-Cuc Phuong National Park; cT-Cat Tien National Park. The Global Positioning System of each site was reported by Le and colleagues (Le *et al.*, 2022).

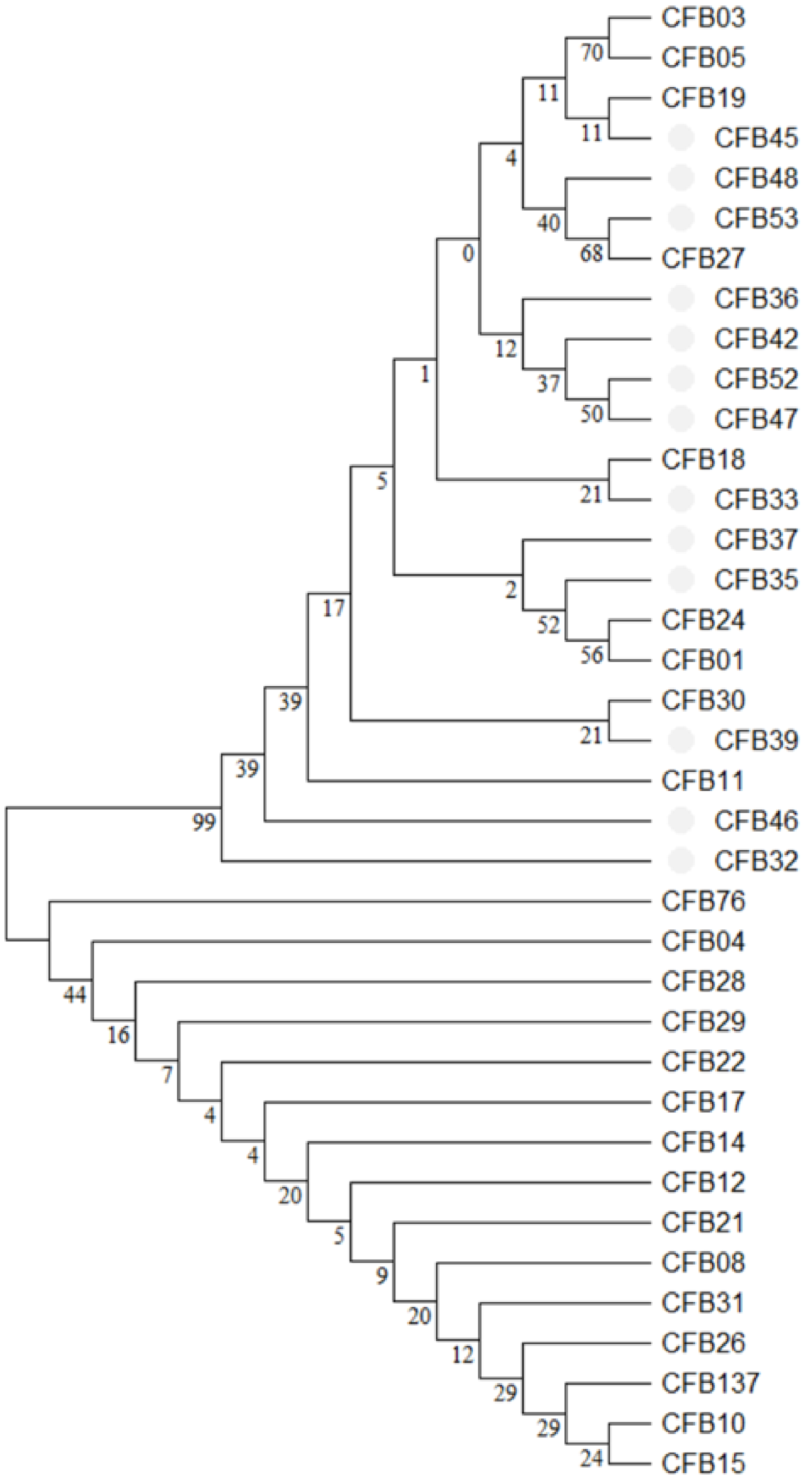

Figure 2. Phylogenetic tree of *Pristionchus* species. The strains from Cat Tien National Park are indicated by circle symbols (●); the rest strains are from Cuc Phuong National Park.

CONCLUSION

We isolated and identified 37 *Pristionchus* strains of possible three species (*P. pacificus*, *P. chinensis,* and a new *Pristionchus* sp. CFB53) from forests. They have potential for research of diversity, and evolution within the genus. To date, the most abundant species is *P. pacificus,* which presents high diversity.

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