

A New Species of *Pristionchus* (Rhabditida: Diplogastridae) and Its Bacterial Symbiont from Yixing, China

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Abstract: A new nematode species, *Pristionchus entomophilus* n. sp., was collected during a soil sample survey in Yixing of Jiangsu province, eastern China. *P. entomophilus* n. sp. is distinguished by its unique characteristics. This new species is mainly hermaphroditic, with males seldom found. The new nematode has a similar body length but has much narrower body width compared with *P. pacificus*. Its body is covered with longitudinal ridges: 12 ridges on head, 13 or 14 ridges in the middle, 11 and 7 ridges in front and rear of the anus, respectively. The eury stomatous form mouth includes a triangular dorsal tooth, a large claw-like right subventral tooth, and a row of five ventral denticles placed opposite the dorsal tooth. Only eight pairs of genital papillae and a pair of phasmids are present in the tail of the male as the sixth pair of papillae having seemingly been degenerated and lost. Molecular phylogenetic trees based on 18S rDNA confirmed that the new species belongs to the genus *Pristionchus* and is most closely related to *P. pacificus*. Moreover, the new species was found to be occasionally associated with the entomopathogenic bacterial strain 09FLYB1 of *Serratia nematodophila* and be able to stably transfer the bacterial strain for several generations.

Key words: insect phoretic, phylogenetic, *Pristionchus*, saprophagous, taxonomy.

Pristionchus pacificus (Kreis, 1932) is an increasingly well-studied molecular genetics satellite organism to the nematode model organism *Caenorhabditis elegans* (Sommer, 2009; Bento et al., 2010). Due to its association with scarab beetles, *P. pacificus* has become a well-established model system in its own right for evolutionary developmental biology (Sommer, 2009; Sommer and Mcgaughran, 2013) that can contribute to the elucidation of diverse issues in evolutionary developmental biology (Eizinger et al., 1999; Félix et al., 2000; Sommer, 2000; Bento et al., 2010). Since *Pristionchus* (Kreis, 1932) was first described, many new species of the genus have been discovered. *Pristionchus* currently contains 48 valid species (Ragsdale et al., 2015), most of which were found to be associated with beetles of genera *Aphodius*, *Cyclocephala*, *Phyllophaga*, *Polyphylla*, *Prismognathus*, and *Variolosia*. However some were discovered associated with other invertebrates in the last few years (Sudhaus and Fürst von Lieven, 2003; Herrmann et al., 2006; Kanzaki et al., 2012a, 2012b, 2013), such as *Pristionchus arcanus* (Kanzaki et al., 2012a) isolated from a termite colony of *Odontotermes* and *P. japonicus* (Kanzaki et al., 2012a) from a dead earthworm.

The genus *Pristionchus* has a wide global distribution with many species found in Asia. *P. fissidentatus* (Kanzaki et al., 2012b) in Nepal, *P. elegans* (Kanzaki et al., 2012b), *P. expectatus* (Kanzaki et al., 2012a), *P. arcanus* (Kanzaki et al., 2012a), *P. japonicus* (Kanzaki et al., 2012a), and

P. bucculentus (Kanzaki et al., 2013) were found in Japan, whereas *P. uniformis* (Herrmann et al., 2006) has been found in the United States and Europe.

During a survey of insectivorous nematodes, we isolated a *Pristionchus* species from a soil sample taken from Yixing, Jiangsu Province, eastern China. We determined it was a new species of *Pristionchus* based on morphological and molecular criteria. The bacteria associated with this worm and its potential infectivity to insects was also examined.

MATERIALS AND METHODS

Nematode isolation and cultivation: Nematodes were obtained from a soil sample collected from Yixing (Jiangsu Province, eastern China) in 2009 by using the insect baiting method (Bedding and Akhurst, 1975; Zhang et al., 2012). Final-instar *Galleria mellonella* larvae were placed in 250-ml plastic containers (five larvae/containers) with moistened soil obtained from the collected sample. Containers were covered with a lid, turned upside down, and kept incubated at constant temperature ($20 \pm 2^\circ\text{C}$) (Stock et al., 1999; Zhang et al., 2012). Water was added to the samples regularly to keep the soil moist during baiting. *G. mellonella* larvae were checked every day, and each dead larva was replaced by a fresh one. After 7 d, dead insects were thoroughly rinsed in distilled water and placed in modified White traps (Kaya and Stock, 1997) until the emergence of nematode dauer larvae. Once the dauer larvae were obtained, 15 *G. mellonella* larvae were exposed to approximately 2,000 nematode dauer larvae in a petri dish (60×15 mm) lined with two moistened filter papers at $20 \pm 3^\circ\text{C}$.

Morphological observation and preparation of type materials: For morphological characterization of the nematode strain, 20 hermaphrodites, 20 males, and 25 dauer larvae were isolated from *G. mellonella* larvae, rinsed three times in distilled water to remove impurities, and heat-killed on glass slides at 60°C in Ringer's

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solution. The heat-killed nematodes were placed in hot triethanolamine–formalin fixative (Kaya and Stock, 1997) and transferred to anhydrous glycerin for mounting (Seinhorst, 1959). Examination and measurements were performed with an AxioImager A1 microscope (Carl Zeiss, Germany). To prepare nematode samples for scanning electron microscopy (SEM) examination, adults and dauer larvae of the new nematode species were fixed in 3% glutaraldehyde buffered with 0.1 M phosphate buffer at pH 7.2 for at least 24 h at 4°C to 8°C (Nguyen and Smart, 1995a), postfixed with 2% osmium tetroxide solution for 12 h at 25°C, dehydrated in a graded ethanol series, critical-point dried with liquid CO₂, mounted on SEM stubs, and coated with gold (Nguyen and Smart, 1995a, 1995b). Samples were examined using a Hitachi S-3000N SEM (Japan).

Molecular and phylogenetic analysis: DNA extraction and polymerase chain reaction (PCR) amplification were performed according to previously described methods (Mayer et al., 2007; Zhang et al., 2008). Primers used for amplification and sequencing of 18S rDNA of the new species were those reported by Liu et al. (1997). The primers for amplification of 18S rDNA were 5'-GGT GAA ACT GCG AAC GGC TCA-3' (forward) and 5'-CCG GTT CAA GCC ATT GCG ATT-3' (reverse). Polymerase chain reaction products were purified and sequenced by Beijing Genomics Institute (BGI, Shenzhen, China). 18S rDNA sequences of *Pristionchus* and related nematodes were used for taxonomic and phylogenetic analyses. DNA sequences of the new species were determined in this study and all others were obtained from GenBank. Sequences were assembled using Sequencing Analysis 3.0 and aligned with ClustalX (Thompson et al., 1997) under the default alignment parameters. Sequences were characterized with MEGA version 5 (Tamura et al., 2011). The program ModelTest 2.0 (Nylander, 2004) was used to find the best fitting substitution model for our molecular dataset. Phylogenetic relationships between these groups were estimated by Bayesian inference methods using MrBayes 3.2.2 (Ronquist et al., 2012). Four cold Metropolis-coupled Markov chains Monte Carlo for 1,000,000 generations were run, and one tree was saved every 100 generations, with the first 2,000 generations discarded as burn-in (Huelsenbeck and Ronquist, 2001).

Isolation and identification of associated bacteria of *P. entomophilus* n. sp.: Associated bacterial strains were isolated from dauer larvae of *P. entomophilus* n. sp. by the method of Akhurst (1980). Approximately 100 surface-sterilized dauer larvae were crushed and then streaked on nutrient bromothymol blue agar plates (nutrient agar, 0.0025% bromothymol blue, and 0.004% triphenyltetrazolium chloride medium) and incubated at 28°C for 48 hr (Akhurst, 1980; Zhang et al., 2012). Genomic DNA of associated bacteria was prepared following the method described by Marmur (1961). Primers used for

amplification and sequencing of these bacterial strains were 5'-AGA GTT TGA TCC TGG CTC AG-3' (forward) and 5'-TAC CTT GTT ACG ACT TCA CCC CA-3' (reverse) (Brosius et al., 1978). Polymerase chain reaction amplification of the 16S rDNA was performed as described by Li et al. (2007). Identification analysis of 16S rDNA sequences of the associated bacterial strains with all described bacteria type strains were conducted using EzTaxon Server version 2.1 (<http://www.ezbiocloud.net/eztaxon>; Chun et al., 2007).

RESULTS

Pristionchus entomophilus n. sp. (Figs. 1–3; Table 1)

Hermaphroditic females: Hermaphroditic females have a C-shaped body when killed by heat. Small females body (length = 807.68 μm and diameter = 43.25 μm), cylindrical, with fine annulations on head and covered with longitudinal ridges: 12 ridges located on head, 13 or 14 ridges in the middle, 11 ridges in front of the anus, 7 ridges to the rear. Boundaries clearly observed. The first boundaries are located near the excretory pore with one longitudinal ridge differentiating into two or three. The second and third boundaries located near the vulva and anus, respectively. Head narrowly rounded, with six lips, each of which has one conical terminal papilla. Only one lip has an extra papilla located behind the conical terminal one. Two pore-like amphids arranged on the lateral sides of two opposite lips. Only eury stomatous form individuals were found in this new species. Cheilostome divided into six distinct parts, constituting a hexagonal opening of the mouth, the edges upturned. Cheilostomal epidermis thin and distinguishable. Three kinds of teeth present, a large triangular dorsal tooth, a large claw-like right subventral tooth, and a row of five left subventral denticles opposite. Dorsal tooth movable and observed in stegostom. Esophagus long and the dorsal pharyngeal gland clearly observed, penetrating dorsal tooth to gland opening. Anterior pharynx muscular and stout, occupying about two-thirds of body width, forming a well-developed median bulb. Isthmus narrow and without muscle. Nerve ring surrounding the isthmus and anteriorly located. Excretory pore emerges on the cuticle near the posterior pharynx. Didelphic and amphidelphic. Anterior gonad located on the right side of the intestine, uterus, and oviduct extending ventrally, posterior gonad antiodromous, reflexed dorsally on the left of the intestine. Oocytes mostly arranged in multiples, two to four rows in the distal two-thirds of the ovotestis, a single row in the remaining third of the middle ovotestis. One to three well-developed oocytes present right at the junction of ovary and oviduct. A well-matured hermaphrodite carries more than 20 eggs. Vulva naked, protrudes slightly in lateral view, appears pore-like in ventral view, situated on a protruding area, usually

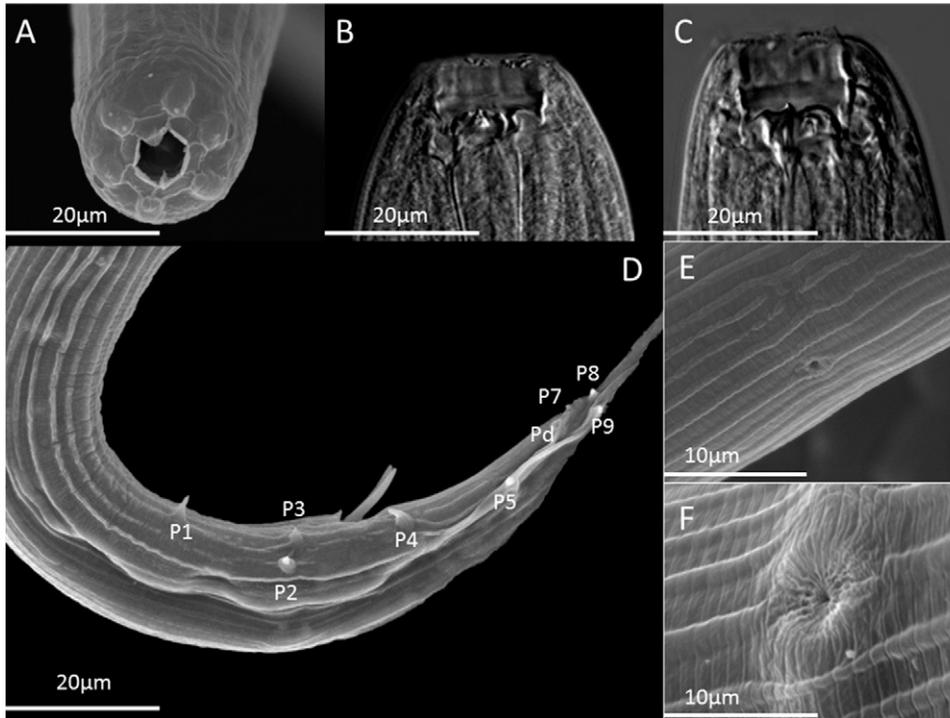


FIG. 1. Scanning electron micrographs (A, D, E, F) and micrographs (B, C) of *Pristionchus entomophilus* n. sp. (A) Head of hermaphroditic female. (B) Dorsal view of mouth of hermaphroditic female showing dorsal tooth and left subventral denticles. (C) Left lateral view of hermaphroditic female showing dorsal tooth and right subventral teeth. (D) Tail of male. (E) Excretory pore of hermaphroditic female. (F) Vulva of hermaphroditic female.

near the mid-body with wrinkled skin. Vagina short and perpendicular to body surface, surrounded by sclerotized tissue. Anus horseshoe shaped with upward bulge in the middle. Rectum about one anal body width in

length; intestinal–rectal junction is surrounded by well-developed sphincter muscle. Three anal glands present. Tail approximately two times longer than the body width at the anus.

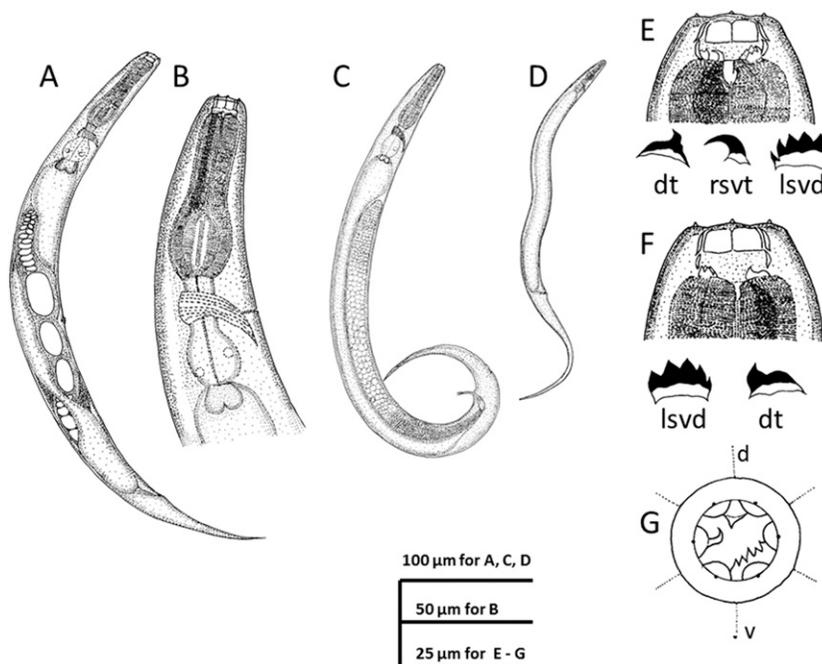


FIG. 2. *Pristionchus entomophilus* n. sp. (A) Mature hermaphrodite in right lateral view. (B) Anterior region of adult in right lateral view. (C) Male in right lateral view. (D) Dauer larva in right lateral view. (E) Left lateral view of eury stomatous form female showing dorsal tooth (dt), right subventral teeth (rsvt), and left subventral denticles (lsvd). (F) Right lateral view of eury stomatous form female. (G) Schematic drawing of face view of eury stomatous form mouth.

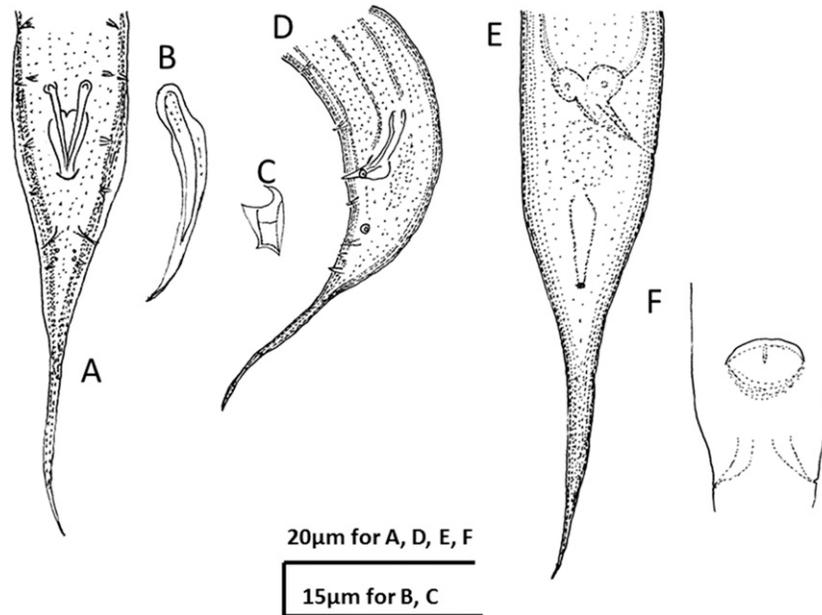


FIG. 3. Tail region in hermaphroditic female and male of *Pristionchus entomophilus* n. sp. (A) Tail region of male in ventral view. (B) Spicule in the left view. (C) Gubernaculum in the left view. (D) Tail region of male in left lateral view. (E) Tail region of female in right lateral view. (F) Anus and phasmids of female in ventral view.

Males: Males have a J-shaped body when killed by heat. Average body length 576.78 μm . Head characteristics are similar to the females. Testis reflexed and monarchic. In the reflexed part, spermatogonia arranged in two to four rows. Mature spermatids near spicules. Vas deferens is not obvious. Gubernaculum boat-shaped (lateral view) and ventrally curved, and approximately one-fifth spicule length. Paired spicules smoothly arcuate ventrally in lateral view, placed symmetrically with about a 30° angle between them. Average spicule length 16.10 μm . Some spicules partially degenerate into projections and buried under the skin. Eight pairs of genital papillae and a pair of phasmids present in the male's tail for the sixth pair of papillae having seemingly been degenerated and lost. Genital papillae distributed in accordance with a 4-1-3 formula. P1, P2, and P3 almost the same size, P2 and P3 are aligned in a row. P4 larger than the three anterior ones. P5 slightly smaller than the other papillae and placed relatively far away from them. P7 and P8 are closely linked. P9 is small but larger than P7 and P8. P7 located in the fluted epidermis, all other genital papillae are on the pedestal. A pair of phasmids located between P5 and P7, but closer to the latter.

Dauer larvae: Nematode dauer larvae have a J-shaped body when killed by heat, with the tail region slightly curved toward the middle portion of the body. Body thin and small, length = 244.30 μm , diameter = 15.79 μm , pharyngeal length = 37.84 μm , two bulb distance = 18.463 μm , tail length = 101.307 μm , distance from anterior end to end of pharynx (ES) = 43.431 μm . Head narrowly rounded, with six lips, with one or two conical terminal papillae. Two amphids, respectively, symmetrical

placed on the two opposite lips. Mouth not clearly open. Posterior pharynx slightly larger than the anterior one. Body cylindrical, fine annulation present near the head, body covered with longitudinal ridges. The esophagus median bulb bulges slightly. The anus is crescent shaped, and the junction of the anus and tail protrudes slightly. The tails of the dauer larvae are covered with distinct ridges. No gonad development. Intestine occupying nearly the entire body.

Type host (carrier) and locality: The natural host is still uncertain. The nematode was originally obtained from soil samples collected from Yixing of Jiangsu province, China.

Type material and nomenclatural registration: Holotypes and paratypes (hermaphroditic females, males, and infective juveniles) were deposited in the Animal Resources Laboratory, Department of Zoology, College of Life Sciences, Nanjing Agricultural University, Nanjing, People's Republic of China. The new species binomial has been registered in the ZooBank database (zoobank.org) under the identifier: 6F8F89B3-0320-4D9D-94DF-4ACF291D6C5C.

Diagnosis and relationships: *Pristionchus entomophilus* n. sp. is distinguished from the phylogenetically closest known species *P. pacificus*, *P. japonicus*, *P. arcanus*, *P. maxplancki*, *P. expectatus*, and other species in the genus *Pristionchus* by its unique morphological characteristics, such as eight pairs of genital papillae existing in its male individuals and the special tooth pattern of its eurytomatic form. *Pristionchus entomophilus* n. sp. (Table 1) is smaller (length = 807.68 μm , diameter = 43.25 μm in hermaphrodite) than *P. pacificus* (length = 1,075 μm , diameter = 81 μm in hermaphrodite) (Sommer, 1996).

TABLE 1. Morphometrics (micrometers) of *Pristionchus entomophilus* n. sp., presented as the mean \pm SD and the range.

Character	Holotype	Male	Hermaphrodite	Dauer larvae
<i>n</i>	1	20	20	20
Body length (L)	584.60	576.782 \pm 52.362 (486.54–704.81)	807.681 \pm 47.879 (733.00–873.13)	244.30 \pm 11.3 (228.82–285.19)
Maximum body diam.	27.14	23.819 \pm 2.399 (20.82–29.35)	43.25 \pm 4.003 (39.39–48.87)	15.79 \pm 1.52 (12.18–17.14)
Stoma (head to tooth)	9.39	9.29 \pm 0.983 (8.34–11.22)	12.90 \pm 1.942 (10.25–14.80)	5.553 \pm 0.473 (4.87–7.08)
Neck length (tooth to anterior pharynx)	62.19	62.917 \pm 6.823 (46.23–73.55)	83.725 \pm 1.861 (80.17–86.34)	37.846 \pm 1.154 (33.27–53.82)
The width of anterior pharynx	14.91	15.435 \pm 1.452 (12.69–17.11)	33.163 \pm 1.773 (31.58–35.13)	12.056 \pm 1.461 (11.76–20.27)
The width of posterior pharynx	12.87	14.044 \pm 1.455 (10.75–16.18)	38.16 \pm 1.649 (36.44–45.12)	16.179 \pm 1.247 (15.83–27.14)
The length of two pharynx	26.99	26.584 \pm 9.003 (14.45–31.89)	55.637 \pm 1.981 (53.02–65.14)	18.463 \pm 1.021 (17.34–29.11)
Tail length with sheath	81.53	91.7925 \pm 17.814 (61.95–129.30)	213.895 \pm 33.130 (170.18–246.98)	101.307 \pm 14.706 (98.84–132.19)
ABD	17.40	15.772 \pm 1.741 (11.95–18.77)	19.275 \pm 1.335 (17.90–21.93)	8.807 \pm 1.844 (7.24–14.51)
ES	62.19	62.916 \pm 6.824 (46.23–73.55)	93.23 \pm 3.258 (89.99–96.41)	43.431 \pm 3.123 (40.85–61.30)
V	—	—	47.978 \pm 0.88 (47.12–49.13)	—
a	21.53	24.483 \pm 2.501 (19.26–26.83)	17.815 \pm 1.063 (16.86–19.03)	17.67 \pm 1.27 (16.18–20.08)
b	9.39	9.254 \pm 1.162 (7.38–12.00)	8.698 \pm 0.169 (8.53–8.91)	8.109 \pm 0.452 (7.34–8.69)
c	7.17	6.458 \pm 1.11 (4.71–9.56)	3.98 \pm 0.988 (3.09–4.88)	4.891 \pm 0.588 (4.22–6.32)
Spicule length	18.71	16.101 \pm 2.620 (12.10–20.47)	—	—
Gubernaculum length	4.16	3.655 \pm 0.537 (2.928–4.825)	—	—

Eight pairs of genital papillae and a pair of phasmids (Figs. 1D; 3A) are present in the tail of the new species' male, distinguishing it from *P. pacificus*, *P. japonicus*, *P. arcanus*, *P. maxplancki*, and *P. expectatus*, which all have nine pairs of genital papillae (Sommer, 1996; Kanzaki et al., 2012a). The special tooth pattern of eurystomatous form (Figs. 1B,C; 2E–G) of the new species is as follow: a large triangular dorsal tooth, a large claw-like right subventral tooth and a row of five ventral denticles opposite the dorsal tooth are located on the metarhabdion of the nematode, which differs substantially from *P. pacificus*, which is composed of a large dorsal retrorse shark-like tooth, a smaller retrorse triangular right subventral tooth visible laterally, and four smaller teeth connected at their base (often only two or three are visible) on the left subventral region of the metarhabdion (Sommer, 1996). The number of left subventral denticles (five) in the new species is different from other *Pristionchus* species, with four denticles in *P. pacificus*, *P. maxplancki*, *P. arcanus*, and *P. expectatus* (Sommer, 1996; Kanzaki et al., 2012a), and six in *P. japonicus* (Kanzaki et al., 2012a).

Molecular and phylogenetic analysis: The obtained length of 18S rDNA of the new nematode species (GenBank ID: KP762794) is 1,670 bp. The best-fitting model was determined as a general time reversible model with a gamma-shaped distribution of rates

across sites (GTR + G) for the 18S rDNA derived from ModelTest (AICc = 7,024.0, BIC = 7,464.8). The phylogeny reconstructed by 18S rDNA gene sequence indicated that the new species has a clear and stable phylogenetic position within the monophyletic *Pristionchus* with the highest posterior probability (1), which suggested that the nematode species should be a member of *Pristionchus* (Fig. 4). *P. pacificus* was the sister group of *P. entomophilus* n. sp. with high posterior probability (0.99), confirming that these two species are closest sister groups among described members of *Pristionchus*. Analysis of all the sequences in the phylogenetic tree in the identity matrix showed that 18S rDNA sequences of *P. entomophilus* n. sp. and *P. pacificus* are the most similar and sharing an identity of 99.3%, whereas those of the new species and *P. elegans* are of 89.9% similarity, which is the most distant relative with the new species.

Isolation and identification of associated bacteria strains: Two representative bacterial strains, 09FLYB1 and 09FLYB2, were isolated from surface disinfected dauer larvae of *P. entomophilus* n. sp. Identification analysis of strain 09FLYB1 with all described bacterial species using 16S rDNA sequences data with EzTaxon Server version 2.1 analysis showed that this strain (GenBank ID: KP765606) belongs to the genus *Serratia* and shares

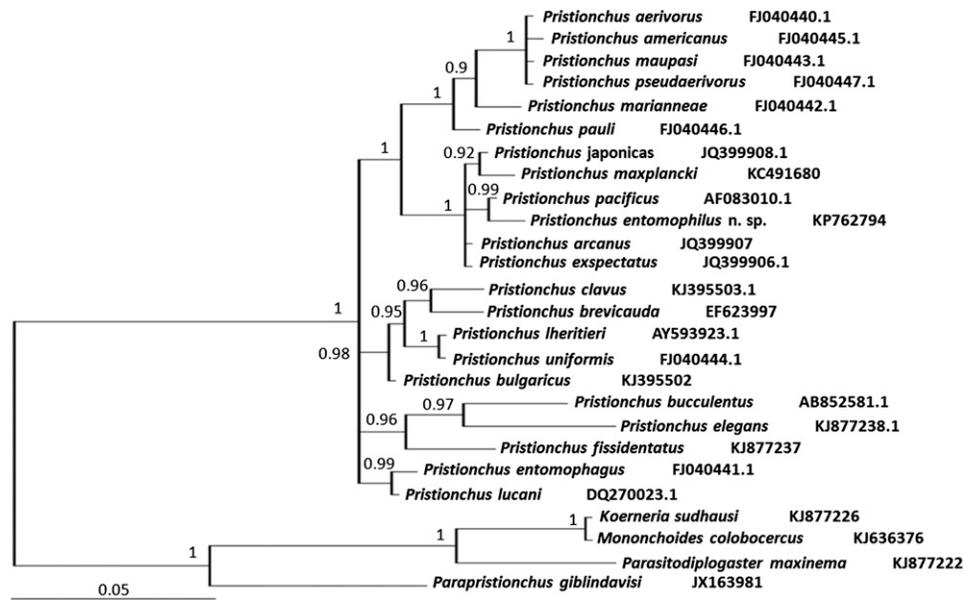


FIG. 4. Phylogenetic relationships of *Pristionchus entomophilus* n. sp. and other closely related nematodes based on 18S rDNA sequences data under GTR + G model (AICc = 7024.0, BIC = 7464.8). Posterior probability values exceeding 50% are given on appropriate clades.

99.855% 16S rDNA sequence similarity with its closer relatives *S. nematodiphila* DZ0503SBS1^T (GenBank ID: EU036987; Zhang et al., 2009) and *S. marcescens* subsp. *sakuensis* KRED^T (GenBank ID: AB061685). Identification analysis of strain 09FLYB2 with EzTaxon Server version 2.1 analysis showed that this strain belongs to the genus *Alcaligenes* and shares 99.399% and 99.398% 16S rDNA sequence similarity with its closer relatives *Alcaligenes faecalis* subsp. *faecalis* IAM12369^T and *A. faecalis* subsp. *phenolicus* J^T, respectively.

DISCUSSION

Phylogenetic analyses of nematodes using 18S rDNA sequences have produced interesting hypotheses that have refined understanding of nematode evolution (Blaxter et al., 1998; Nadler and Hudspeth, 1998; Blaxter, 2001). 18S rDNA sequences data have revealed the phylogenetic position of many new nematode species and addressed some controversies about their taxonomy (Zhang et al., 2008, 2012). This study identified a novel nematode species, *P. entomophilus* n. sp. The new species is morphologically similar to *P. pacificus*, but can be clearly distinguished from it in 18S rDNA sequences, and in some abovementioned morphological characteristics, such as the male's genital papillae pattern, and some morphometric characteristics. Phylogenetic trees of 18S rDNA constructed in this study showed that the new nematode is most closely related to *P. pacificus* among the monophyletic genus *Pristionchus*, with these two species comprising a sister group. Sequence of *P. entomophilus* n. sp. and *P. pacificus* are closest with a similarity of 99.3% and *P. entomophilus* n. sp. shares 98.8 % similarity with its closer relatives *P. arcanus* and

P. exspectatus. Only eurytomatous form individuals were found in this new species (Figs. 1B,C; 2E–G), but this is environmentally malleable in *Pristionchus*, and both forms are likely on further investigation (Kiontke and Fitch, 2010; Wilecki et al., 2015).

The new species was originally isolated from soil samples collected from Yixing of Jiangsu province, China, with the *G. mellonella* final-instar larva baiting method. This is the first described species in the genus *Pristionchus* that can infect live lepidopteran larvae and reproduce successfully. Most previously described species of *Pristionchus* are found in association with beetles; *P. exspectatus* was isolated from an adult of *Prismognathus*, and *P. pacificus* was found repeatedly on five different scarab beetles, *Odontotermes*, *Adoretus*, *Maladera*, *Hoplia*, and *Hoplochelus* (Herrmann et al., 2010). Some species were found associated with other animals, such as colonies of *Odontotermes formosanus* and dead earthworms (Kanzaki et al., 2012a, 2012b). However, these morphological and host preference characteristics are not the only ones we found that distinguish the new species from previously described members of *Pristionchus*.

We repeatedly collected soil samples in the same site where we isolated *P. entomophilus* n. sp. A population of the new species isolated from the first collected sample was found to be associated with two bacterial strains 09FLYB1 and 09FLYB2. Identification analysis of strain 09FLYB1 with EzTaxon Server version 2.1 showed that this strain shares 99.855% 16S rDNA sequence similarity with its closer relatives *Serratia nematodiphila* DZ0503SBS1^T (Zhang et al., 2008) and *S. marcescens* subsp. *sakuensis* KRED^T (Ajithkumar et al., 2003). *Serratia nematodiphila* was previously shown as the bacterial

symbiont of the two rhabditid entomopathogenic nematodes (EPNs) *Heterorhabditidoides chongmingensis* (Zhang et al., 2008, 2009) and *H. rugaoensis* (Zhang et al., 2012). This marks the first discovery that a nematode species of the genus *Pristionchus*, could be associated with an EPN bacterial symbiont. Further studies should focus on the construction mechanism of the occasionally symbiotic relationship between *Pristionchus* and *Serratia* along with the bacterial toxicity. To some extent, species of *Pristionchus* that can host EPN symbiotic bacteria will enrich the catalogue of EPNs, and provide a safe, efficient alternative resource for dealing with agriculture and forestry pests.

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