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# Morphological and molecular characterisation of *Paralongidorus sacchari* (Nematoda: Longidoridae), a new record of needle nematode in China

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**Abstract** A needle nematode of the genus *Paralongidorus* was isolated from Cuban royal palm (*Roystonea regia*) in China. Detailed morphological study confirmed that this species was the described needle nematode *P. sacchari*. Accordingly it was formally described and photographed. This nematode is characterised by a long body (4348–5825 μm), a board and anteriorly flat lip region slightly offset from body contour, bearing a stirrup-shaped amphidial fovea, with conspicuous slit-like aperture, a long and flexible odontostyle *ca* 101–110 μm long, stylet guiding ring located at 27.5–33.0 $\mu$m from anterior end, vulva near mid-body (48.0–50.0%), a short dorsally convex tail, with rounded terminus, and male absent. Molecular characterisation using near full-length 18S rRNA and D2-D3 expansion segments of 28S rRNA gene was also provided. Phylogenetic trees inferred from BI analysis of the two rRNA gene fragments revealed that *P. sacchari* could be distinguished from all described needle nematodes with molecular data, as well as the closely related species *P. bikanerensis* and *P. sali*. This nematode is a new record of *Paralongidorus* species from China. Cuban royal palm is a new host plant for *P. sacchari*.

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**Keywords** Morphology · New record · Phylogeny · Needle nematode · Cuban royal palm

## Introduction

The Genus *Paralongidorus* was erected by Siddiqi et al. (1963) with two new species, *P. sali* Siddiqi et al., 1963 and *P. sacchari* Siddiqi et al., 1963. The genus *Paralongidorus* morphologically resembles *Longidorus*, but differs from the latter by shape of amphidial fovea (stirrup-shape, open goblet-shaped, open goblet-shaped, funnel-shaped, elongate stirrup-shaped or pouch-like vs pouch-like only) and amphidial opening (transverse slit-like vs pore-like) (Decraemer and Coomans 2007).

Like the genus *Longidorus*, *Paralongidorus* species are also migratory ectoparasites of plant roots (Decraemer and Robbins 2007). *Paralongidorus* species causes direct damage to a variety of host plant by feeding activity and one species, *P. maximus*, is also vectors of plant pathogenic viruses (Decraemer and Robbins 2007). Therefore, *P. maximus* is paid more attention to because its quarantine importance in many countries including China (Taylor and Brown 1997; Decraemer and Robbins 2007; Meador and Wu 2011).

Some 90 species of *Paralongidorus* have been recorded (Decraemer and Robbins 2007). As the number of nominal species increases, morphological identification of *Paralongidorus* species is a challenging task for the obvious interspecific overlapping and significant intraspecific variability of some diagnostic characters.

Therefore, molecular techniques such as comprehensive analyses of fragments of rRNA genes are recommended to the identification of *Paralongidorus* spp. (Palomares-Rius et al. 2008, 2013; Pedram et al. 2012; Barsi and Luca 2017; Gutiérrez-Gutiérrez et al. 2018). In the past few years, several *Paralongidorus* species, i.e. *P. litoralis* Palomares-Rius et al., 2008, *P. iranicus* Pedram et al., 2012, *P. plesioepimikis* Palomares-Rius et al., 2013, *P. francolambertii* Barsi and Luca, 2017, *P. lusitanicus* Gutiérrez-Gutiérrez et al., 2018 were proposed as new species based on morphological characters and molecular approaches (Palomares-Rius et al. 2008, 2013; Pedram et al. 2012; Barsi and Luca 2017; Gutiérrez-Gutiérrez et al. 2018). However, molecular data of most *Paralongidorus* species are not currently available.

During 2015–2017, extensive surveys of plant nematodes on ornamental trees and shrubs were done in Guangdong Province. One population of *Paralongidorus* was found in a loamy soil in the rhizosphere of Cuban royal palm in China. Detailed morphological and molecular comparative study using previously reported data combined with molecular analyses showed that the population differed from all known *Paralongidorus* species except *P. sacchari*.

The objectives of this work were to: (i) characterize morphologically and molecularly *P. sacchari* from China; and (ii) study the phylogenetic relationships of this species with other *Paralongidorus* spp. and *Longidorus* spp. using sequences from near full-length 18S rRNA gene and D2-D3 expansion segments of 28S rRNA gene as inferred from Bayesian inference (BI) approaches.

## Materials and methods

### Nematode population and morphological studies

Twelve soil samples around the roots of Cuban royal palm were collected from different sites, Guangzhou, Guangdong province, China. For every sample, about 20 cm-depth topsoil was taken by stainless steel sampling tube. Needle nematodes of the genus *Paralongidorus* were isolated from soils by decanting and sieving method (Brown and Boag 1988). Needle nematodes were only detected in one sample from Tianhe district (isolate DWY: 23°9′ 47.1“ N, 113°21’ 21.7” E, 35 m a.s.l.). 34 nematodes of different stages were obtained from *ca* 200 mL soil.

Fresh nematodes were gentle heated, fixed in 4% formaldehyde and processed to pure glycerin (Seinhorst 1959). Nematodes from permanent slides were photographed and measured under a Nikon ECLIPSE Ni microscope (Nikon, Tokyo, Japan). A polytomous key from Escuer and Arias (1997) was used for species identification of the genus *Paralongidorus*.

### DNA extraction, amplification and sequencing

DNA was extracted from individuals of female according to the method described by Mundo-Ocampo et al. (2008). Two rRNA gene fragments, i.e., near full-length 18S rRNA gene and D2-D3 expansion segments of 28S rRNA gene, were amplified from three specimens respectively. Primers for near full-length 18S rRNA gene amplification were 988F (5′-CTC AAA GAT TAA GCC ATG C-3′), 1912R (5′-TTT ACGGTC AGA ACT AGG G-30), 1813F (5′-CTG CGT GAG AGG TGA AAT-3′) and 2646R (50-GCT ACC TTG TTA CGA CTT TT-3′) (Holterman et al. 2006). Primers for D2-D3 expansion segments of 28S rRNA gene amplification were D2A (5’-ACA AGT ACC GTG GGG AAA GTT G-3′) and D3B (5’-TCG GAA GGA ACC AGC TAC TA-3′) (De Ley et al. 1999). Detailed protocols of PCR amplification were conducted as described by previous study (De Ley et al. 1999; Holterman et al. 2006). DNA fragments were sequenced as described in Zhuo et al. (2010). The newly obtained sequences were deposited in the GenBank database and the accession numbers are MH973643-MH973645 and MK920217-MK920220.

### Phylogenetic analysis

The sequences of *P. sacchari* were compared with needle nematode sequences in GenBank using Standard Nucleotide BLAST (blastn) program. The close-related and published sequences of needle nematodes were selected for phylogenetic analyses. Outgroup taxa for each dataset were chosen according to previous phylogenetic study for needle nematodes (Pedram et al. 2012; Palomares-Rius et al. 2013). DNA sequences were aligned by ClustalW implemented in MEGA6.0 (Tamura et al. 2013) using default parameters. Nucleotide substitution models were evaluated using MODELTEST3.7 (Posada and Crandall 1998)

combined with PAUP4.0 (Swofford 1998). The Akaike-supported model, the base frequencies, the proportion of invariable sites, the gamma distribution shape parameters and substitution rates were used in phylogenetic analyses. Bayesian analysis for both genes under the GTR + I + G model, was employed to confirm the tree topology using MrBayes 3.2 (Huelsenbeck and Ronquist 2001) running four chains for $1 \times 10^6$ generations and setting the 'burn-in' at 2500. The MCMC (Markov Chain Monte Carlo) method was performed within a Bayesian framework to estimate the posterior probabilities of the phylogenetic trees (Larget and Simon 1999) and generate a 50% majority rule consensus tree. TREEVIEW1.6 was used to display and edit the trees (Page 1996).

## Results

### Description of the needle nematode *Paralongidorus sacchari*

Measurements of females are listed in Table 1. Illustrations and photos are in Figs. 1, 2, and 3.

#### Female

Body medium long, tapering gradually toward anterior end, open C-shaped when killed by gentle heat, more curved ventrally in posterior half (Fig. 2a). Cuticle appearing smooth under light microscope, 3.0–4.0 μm thick along the body, 7.5–9.2 $\mu$m thick at tail end. Lip region board, 14.4–15.6 $\mu$m wide, more than 2 times as long as high, anteriorly flat, continuous with body contour (Fig. 2: d-e). Amphidial fovea funnel-shaped or stirrup-shaped (Fig. 2e), with broad slit-like aperture, almost as wide as lip region (Fig. 2b). Odontostyle long and narrow, 1.5–1.9 times as long as odontophore, straight or slightly arcuate, odontophore weakly developed, with slightly swollen base (Fig. 2c). Stylet guiding ring single, 1.8–2.2 times head widths from anterior end. Nerve ring encircling narrower part of pharynx, slightly posterior to odontophore base. Anterior slender part of pharynx usually with looped region overlapping basal bulb, basal bulb cylindrical, 113–139 μm long, 21.0–29.5 $\mu$m diameter, 4.3–5.7 times as long as wide (Fig. 2f). Cardia conoid-rounded, 8–16 μm long. Dorsal pharyngeal gland nucleus in anterior part of bulb, 25.5–33.0 μm posterior to gland outlet, one ventrosublateral pair of nuclei near middle of bulb, 63.5–73.5 $\mu m$ posterior to gland outlet (Fig. 2f). Vulva in form of a transverse slit, located at or slightly anterior to mid-body, vagina slopping backward, occupying 58.0–63.5% of corresponding body width, *pars distalis vaginae* and *pars proximalis vaginae* 16.5–18.5 μm and 15.5–18.5 $\mu$m long, respectively. Reproductive system with both genital branches almost equally developed. Anterior and posterior genital branches 321–424 $\mu$m and 253–302 $\mu$m long, respectively, with reflexed ovaries and single raw of oocytes (Fig. 2g). Well developed sphincter between oviduct and uterus. Prerectum 313–470 μm long and rectum 21–26.8 $\mu$m long. Tail short, 0.9–1.3 anal body diam. Long, dorsally convex, with rounded terminus (Fig. 2: h-j), bearing two caudal pores on each lateral side(Fig. 2k).

#### Male

Not found.

#### Juveniles

All four juvenile stages were found and distinguished by relative lengths of body and functional and replacement odontostyle (Table 1; Fig. 3: a-i), resembling adults in most respects except for size and development of reproductive system, more elongate and differently shaped tail (Fig. 3: j-m). First-stage juveniles ($J_1$s) characterized by an elongate-conoid tail (Fig. 3m), odontostyle length 48–60 $\mu$m long (Fig. 3i), and shorter distance from anterior end to stylet guiding ring (Fig. 3i) than that in adult stages. However, morphology in all four juvenile stages (except for undeveloped genital structures) similar to that of female, including broadly rounded tail shape of fourth-stage juveniles, yet differed in shorter distance from anterior end to guiding ring (Fig. 3).

#### Hosts and localities

A population of *P. sacchari* extracted from rhizosphere of Cuban royal palm (*Roystonea regia* O.F.Cook) collected in Tianhe district, Guangzhou, Guangdong Province, China, in October, 2015. It has been recorded from the type locality in Australia from soils around the roots of sugarcane (*Saccharunt officinarum*) (Siddiqi

**Table 1** Morphometrics of females and juveniles of *Paralongidorus sacchari* population from China. All measurements are in $\mu$m and in the form: mean ± s.d. (range)

| Character | Female | J4 | J3 | J2 | J1 |
|---|---|---|---|---|---|
| n | 12 | 5 | 5 | 6 | 5 |
| L | 4984 ± 471 (4348–5825) | 3690 ± 361 (3304–4232) | 2692 ± 266 (2440–3117) | 1863 ± 165 (1714–2129) | 1235 ± 52 (1165–1299) |
| a | 85.5 ± 6.9 (78.0–102.0) | 78.5 ± 7.0 (69.5–88.0) | 66.0 ± 3.5 (62.5–71.0) | 61.5 ± 7.0 (49.5–68.5) | 55.5 ± 5.0 (52.0–64.0) |
| b | 11.1 ± 1.1 (9.7–12.8) | 8.9 ± 1.2 (7.5–10.7) | 8.3 ± 1.3 (7.2–9.7) | 6.3 ± 0.6 (5.5–7.0) | 5.0 ± 0.6 (4.3–5.8) |
| c | 136.0 ± 17.5 (108.0–165.0) | 87.0 ± 6.5 (78.0–94.0) | 69.0 ± 8.5 (58.5–81.0) | 42.5 ± 5.0 (38.0–52.0) | 28.5 ± 3.0 (24.0–32.0) |
| c’ | 1.0 ± 0.1 (0.9–1.3) | 1.3 ± 0.1 (1.2–1.4) | 1.5 ± 0.1 (1.4–1.6) | 2.2 ± 0.2 (1.9–2.4) | 3.0 ± 0.2 (2.7–3.3) |
| V | 49.0 ± 1.0 (48.0–50.0) | | | | |
| Lip region diam. | 15.0 ± 0.5 (14.5–15.5) | 13.2 ± 0.2 (13–13.4) | 12.2 ± 0.6 (11.8–12.6) | | |
| Lip region height | 6.9 ± 0.4 (6.5–7.5) | 6.0 ± 0.1 (5.9–6.1) | 5.4 ± 0.4 (5.0–5.7) | | |
| Odontostyle | 105 ± 3 (101–110) | 92 ± 4 (87–97) | 79 ± 5 (71–85) | 64 ± 3 (62–70) | 55 ± 6 (48–60) |
| Odontophore | 64 ± 3 (58–67) | 57 ± 4 (51–60) | 52 ± 5 (48–59) | 52 ± 5 (44–58) | 42 ± 3 (38–45) |
| Total stylet | 169 ± 4 (160–176) | 149 ± 7 (140–157) | 133 ± 8 (124–144) | 114 ± 8 (106–128) | 94 ± 8 (84–103) |
| Replacement odontostyle | | 105 ± 6 (99–114) | 89 ± 3 (86–93) | 78 ± 3 (74–83) | 66 ± 3 (63–68) |
| Guide ring | 30.5 ± 1.5 (27.5–33.0) | 26.5 ± 1.5 (25.5–29.5) | 23.0 ± 1.0 (22.5–24.5) | 20.5 ± 1.5 (18.5–21.5) | 17.5 ± 0.5 (16.5–18.0) |
| Pharyngeal bulb length | 125 ± 8 (113–139) | | | | |
| Pharyngeal bulb diam. | 24.5 ± 2.5 (21.0–29.5) | | | | |
| Anterior genital branch | 362 ± 55 (321–424) | | | | |
| Posterior genital branch | 273 ± 26 (253–302) | | | | |
| Anterior genital branch (%) | 7.0 ± 1.4 (5.8–8.5) | | | | |
| Posterior genital branch (%) | 5.3 ± 0.7 (4.7–6.1) | | | | |
| Body diam. at mid body | 58.5 ± 5.0 (53.0–72.5) | 45.5 ± 3.5 (41.0–49.5) | 41.0 ± 5.0 (36.0–47.5) | 30.5 ± 4.0 (26.5–38.5) | 22.0 ± 1.5 (20.5–24.0) |
| Body diam. at anus | 36.0 ± 2.5 (31.5–39.5) | 32.0 ± 2.5 (29.5–35.5) | 26.0 ± 1.5 (24.5–28.0) | 20 ± 1.5 (18.5–21.5) | 15.0 ± 1.0 (13.5–16.0) |
| Rectum | 23.0 ± 3.5 (21.0–27.0) | | | | |
| Tail | 37.0 ± 3.0 (32.5–40.0) | 42.0 ± 1.5 (40.0–44.0) | 39.0 ± 2.0 (36.5–41.5) | 44.5 ± 3.5 (41–50.0) | 43.5 ± 5.5 (38.5–51.0) |
| Hyaline tail tip | 8.3 ± 0.6 (7.5–9.2) | | | | |
| Prerectum | 382 ± 81 (313–470) | | | | |

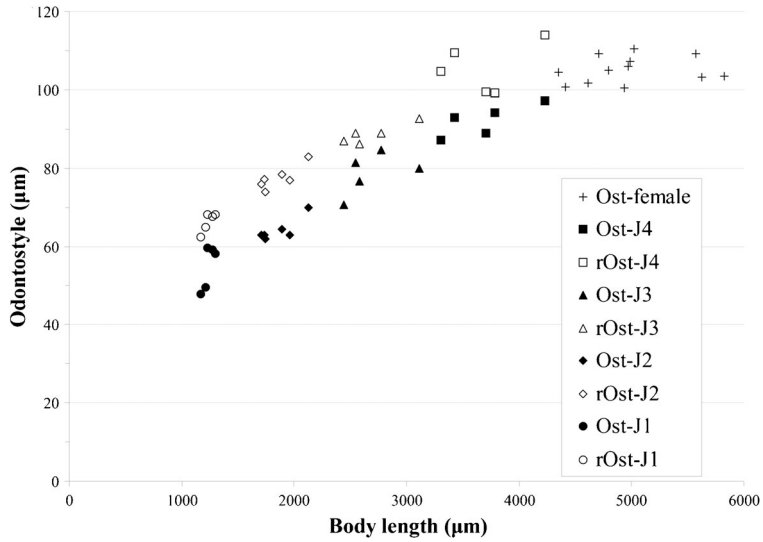


**Fig. 1** Relationship of body length with length of functional and replacement odontostyle (Ost and rOst, respectively) in all detected developmental stages to mature females of *Paralongidorus sacchari* population from China

et al. 1963). Other populations are discovered from sugarcane soil in India (Siddiqi et al. 1963) and soil around roots of *Jubaea chilensis* in Chile (Roca and Rios 2006).

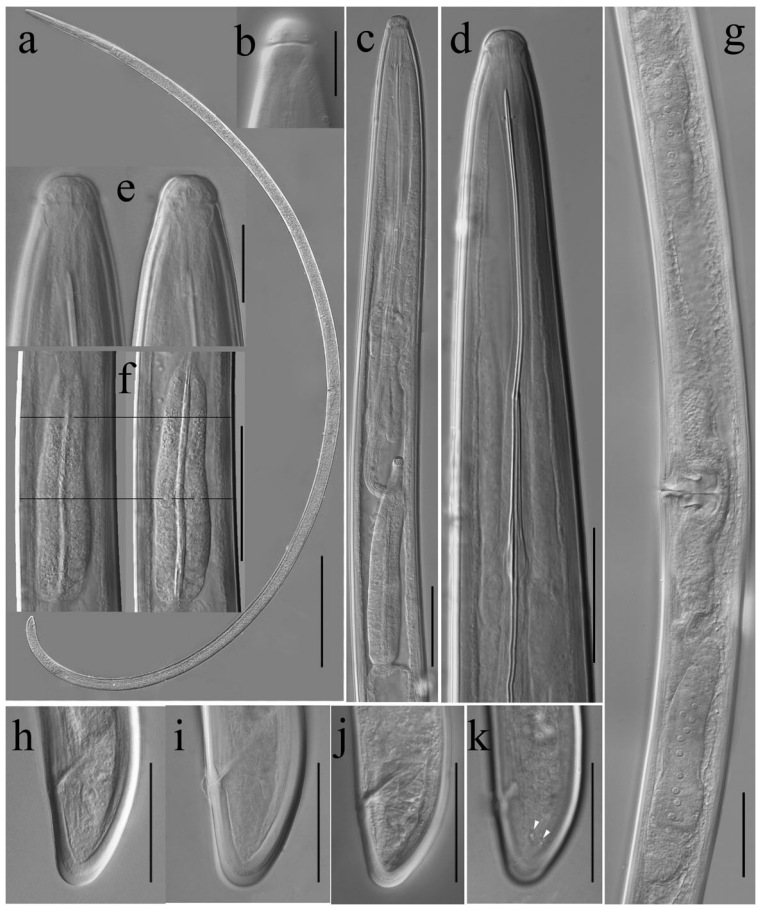


**Fig. 2** Females of *Paralongidorus sacchari* population in China under the light microscope. **a** entire body; (**b**) amphidial aperture; (**c**) pharyngeal region; (**d**) lip region and stylet; (**e**) amphidial fovea; (**f**) pharyngeal bulb; (**g**) vulval region and ovary; (**h-i**) tails (arrows show caudal pores). (Scale bars: **a** = 500 µm; **b, e** = 20 $\mu$m; **c-d, f-k** = 50 $\mu$m)

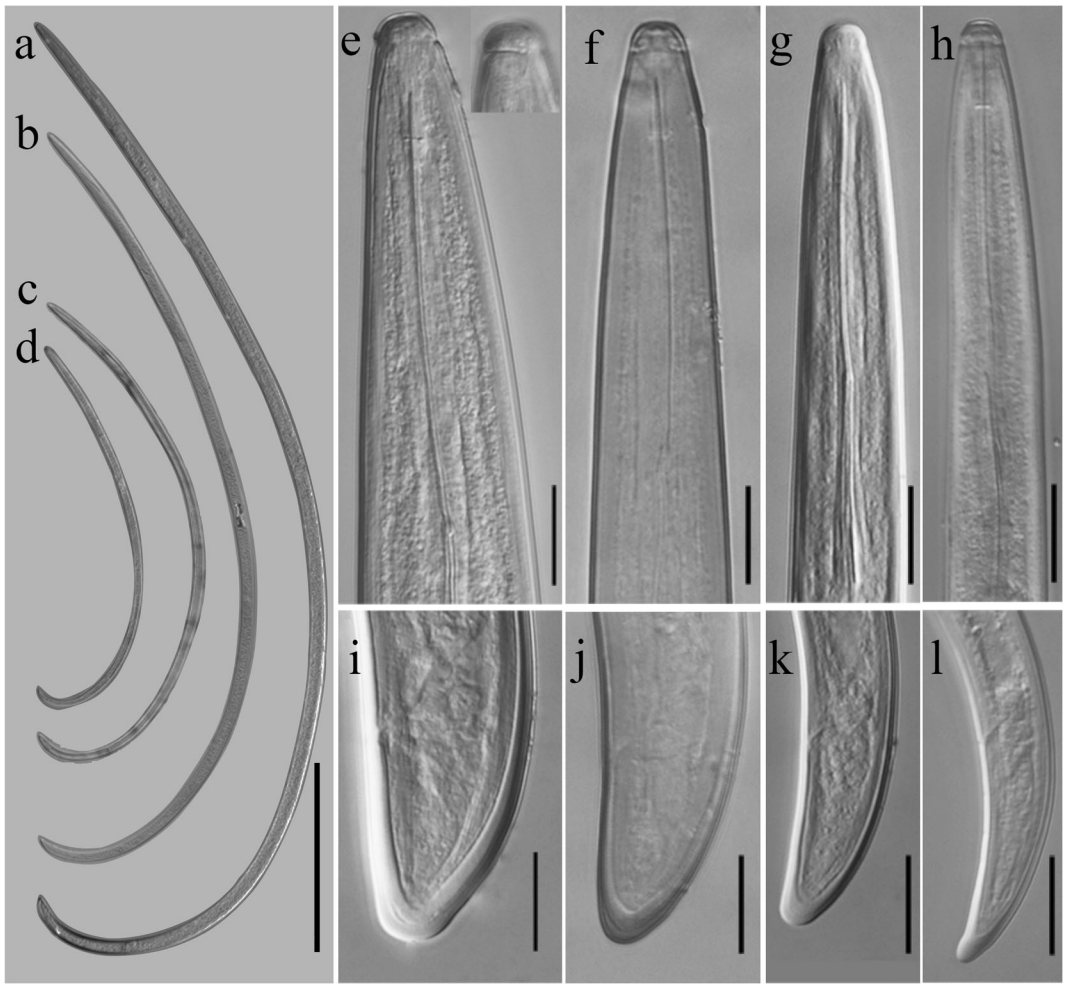


**Fig. 3** Juveniles of *Paralongidorus sacchari* population in China under the light microscope. (**a-d**) entire body of J1, J2, J3 and J4, respectively; (**e**) anterior region of J4; (**f**) amphidial fovea of J4; (**g-i**) anterior region of J3, J2 and J1, respectively; (**j-m**) tails of J4, J3, J2 and J1, respectively. (Scale bars: **a-d** = 200 μm; **e-m** = 20 μm)

Remarks

Females and Juveniles of population from China are morphologically coinciding with those from the type population from Australia, with minor difference in the reproductive system structures (genital branches asymmetrical in the Chinese population vs symmetrical in the type population). Females from China and Australia resemble those from India except shorter odontostyle length (101–110 μm and 105–114 μm vs 116–120 μm from India). Females from China and Australia are similar with those of population from Chile with an exception of some variations in three characters: wider lip region diameter [14.5–15.5 μm and *ca* 16 μm (inferred from illustration) vs 9.5–11.5 μm from Chile], more anterior position of the guide ring (27.5–33.0 μm and 30–33 μm vs 36–43 μm from Chile) and larger pharyngeal bulb size (113–139 × 21–29.5 μm and 127 × 22 μm vs 88–107 × 16–20 μm from Chile). First-stage juveniles ($J_1$s) from China and Australia differ from those from Chile by shorter odontostyle (48–60 μm and 47–56 μm vs 62–74 μm from Chile), shorter replacement odontostyle (63–68 μm and 63–67 μm vs 73–94 μm from Chile), and more anterior position of the guide ring (16.5–18.0 μm and 16–18 μm vs 23–27 μm from Chile).

*Molecular characterisation*

A near full-length 18S rRNA gene of *ca* 1630 bp was obtained from *P. sacchari* population in China. Intra-population variations for *P. sacchari* China population

were 0–0.1% (0-2 bp difference in compositon). A Blastn search of the 18S rRNA gene sequences showed 99.1%–99.3% similarities with *P. bikanenrensis* (JN032586) and 98.9%–99.3% similarities with *P. sali* (MG729696-MG729697). 18S rRNA gene sequences of *P. sacchari* differ from that of *P. bikanerensis* in 12 bp −14 bp, and from those of *P. sali* in 12 bp −19 bp. A phylogenetic tree (Fig. 4) based on near full-length 18S rRNA gene was from a multiple alignment of 1643 total characters with 1408 constant characters (85.7%). The average nucleotide composition was as follows: 26.71% A, 20.75% C, 26.51% G and 26.02% T. In the 18S rRNA gene trees, all *Paralongidorus* species with molecular data clustered into two separate groups. *P. sacchari* formed a clade with *P. bikanerensis* (JN032586) and *P. sali* (MG729696-MG729697) but with low support (PP = 51). Other species including *P. litoralis* (EU026158), *P. lusitanicus* (KY750569), *P. plesioepimikis* (JQ673405), *P. paramaximus* Heyns, 1965 (EU026157), *P. maximus* (Bütschli, 1874) Siddiqi 1964 (AJ875152), *P. iranicus* (JN032589) and *P. rex* Andrássy, 1986 (KJ427794) formed a major high support clade (PP = 100). The position of *L. laevicapitatus* Williams, 1959 (KX136873) was in a basal position of the BI tree.

Amplification of D2-D3 expansion segments of 28S rRNA gene from *P. sacchari* population in China yielded a single fragment of *ca* 850 bp. Intra-population variations in D2-D3 expansion segments of 28S rRNA gene sequences for *P. sacchari* China population were 0–0.1% (0-1 bp difference in length). A Blastn search of the 18S rDNA sequences showed 85.7% similarity with *P. bikanenrensis* (JN032584) and 84.8%–85.1% similarities with *P. sali* (MG729700-MG729701). D2-D3 expansion segments of 28S rRNA gene of *P. sacchari* differs from that of *P. bikanerensis* in 117 bp, and from those of *P. sali* in 122 bp −128 bp. A phylogenetic tree (Fig. 5) based on D2-D3 expansion segments of 28S rRNA gene was from a multiple alignment of 849 total characters with 331 constant characters (39.0%). The average nucleotide composition was as follows: 24.29% A, 21.55% C, 27.45% G and 26.71% T. Similar to the 18S rRNA gene trees, all sequences of *Paralongidorus* species also formed two separate groups in BI trees of D2-D3 expansion segments of 28S rRNA gene. *P. sacchari*, *P. bikanerensis* (JN032584) and *P. sali* (MG72970-MG729701) clustered together but with relative long genetic distance (PP = 100). The major clade contained *P. rex* (AY601582 and KJ427793), *P. francolambertii* (LT669805), *P. iranicus* (JN032587), *P. maximus* (AF480083), *P. litoralis* (EU026155), *P. paramaximus* (EU026156), *P. plesioepimikis* (JQ673403) and *P. lusitanicus* (KY750562), and received strong support (PP = 100). The position of *L. laevicapitatus* (KX136865) was in a basal position of the BI tree.

## Discussion

In this study, *P. sacchari* is closely related to *P. bikanerensis* molecularly and phylogenetically. However, *P. sacchari* can be distinguished morphologically from *P. bikanerensis* by more posterior vulva position (V = 48.0–50.0 vs V = 43–47), shorter odontostyle and odontophore (101–110 μm and 58–67 μm vs 121–132 μm and 66–76 μm, respectively), more anterior position of the guide ring (27.5–33.0 μm vs 32.5–37.4 μm), shorter tail hyaline region (7.5–9.2 μm vs 10.5–14.0 μm), larger c' value (2.7–3.3 vs 2.5–2.6) of $J_1$, and shorter $J_1$ replacement odontostyle (63–68 μm vs 74.5–77 μm).

According to the original description, the amphidial fovea of *P. sacchari* was described as funnel-shaped. Female specimens of *Paralongidorus* with different orientations of the body might reveal a different shape of the fovea (Decraemer and Coomans 2007). Decraemer and Coomans (2007) examined some paratype females of *P. sacchari* and the amphidial fovea was considered to be stirrup-shaped. For the Chinese population of *P. sacchari*, one side of amphidial fovea wall is straight and the other side is curve. Therefore, the shape of the amphidial fovea for *P. sacchari* China population is between funnel-shaped and stirrup-shaped.

The phylogenetic relationship between *Paralongidorus* and *Longidorus* is still not clear. In both trees, two separate *Paralongidorus* groups are nested into *Longidorus* spp., and *L. laevicapitatus* was in a basal position. Both *Paralongidorus* and *Longidorus* are not monophyletic groups (Pedram et al. 2012; Palomares-Rius et al. 2013). *Paralongidorus* was even not accepted as a valid taxon (Gutiérrez-Gutiérrez et al. 2011).

Taxonomy status of the genus *Longidoroides* Khan et al., 1978 was controversial (Siddiqi et al. 1993; Coomans 1996; Escuer and Arias 1997). Siddiqi et al. (1993) synonymised *Longidoroides* with

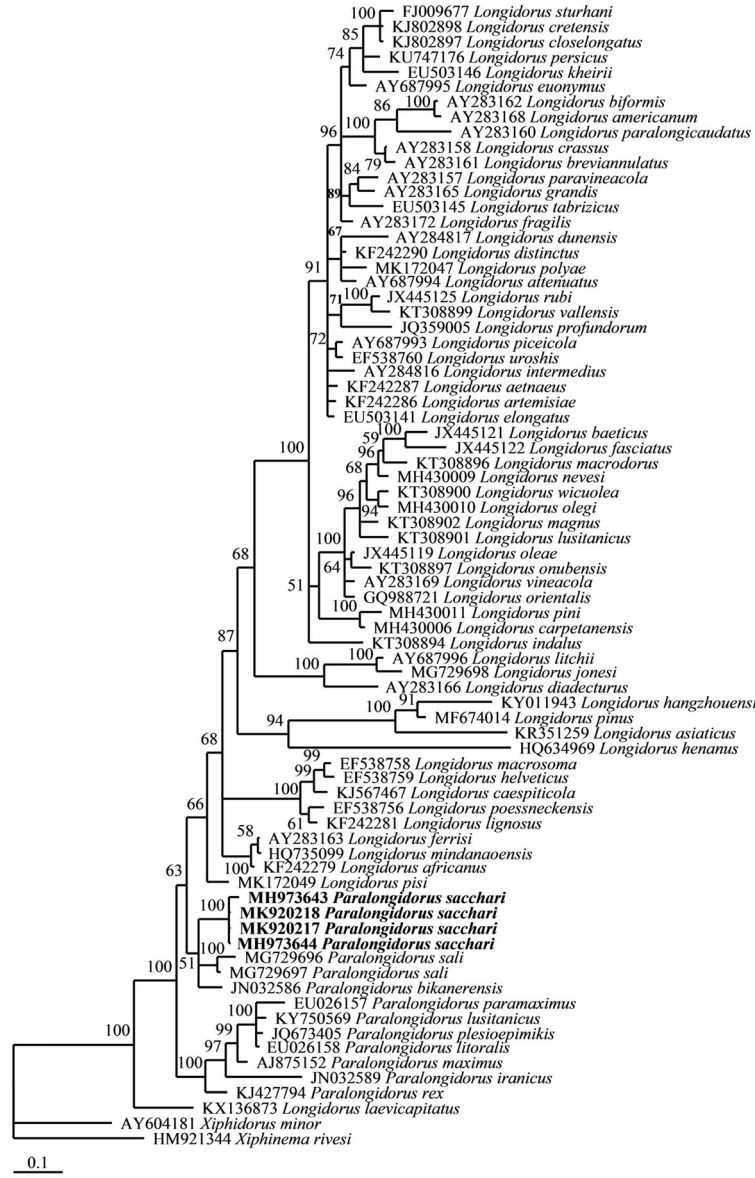


**Fig. 4** Bayesian consensus tree inferred from near full-length 18S rRNA gene of *Paralongidorus sacchari* population in China under GTR + I + G model (lnL = 6014.6235; AIC = 12,049.2471; freqA = 0.2671; freqC = 0.2075; freqG = 0.2651; freqT = 0.2602; R(a) = 1.7119; R(b) = 3.9143; R(c) = 2.3649; R(d) = 0.5176; R(e) = 7.7103; R(f) = 1; Pinva = 0.7565; Shape = 0.5218). Posterior probability values exceeding 50% are given on appropriate clades. Newly obtained sequences are indicated in bold

*Paralongidorus*, but this viewpoint was not admitted by Coomans (1996). *P. bikanerensis*, a member of former *Longidoroides* species, was the only species outside the major group of *Paralongidorus* in all phylogenetic trees reported in previous studies (Pedram et al. 2012; Palomares-Rius et al. 2013). *P. sacchari* showed close relationship with *P. bikanerensis* and *P. sali* in both trees, which further demonstrated the taxonomy status of *Longidoroides* as synomyn of *Paralongidorus*.

The Genus *Longidorus*, which is widely distributed in China, comprises about 18 species (Barsalote et al. 2018; Xu et al. 2018). However, the genus *Paralongidorus* was seldom discovered in China. *P. sali* collected from the rhizosphere of woody perennials, was the first *Paralongidorus* species recorded from China until 2018 (Cai et al. 2018). However, *Paralongidorus* species are very abundant in India (34 spp.), a neighboring country of China (Decraemer and Robbins 2007). The origin centre for *Paralongidorus* may be located in the region of South-East Africa to India (Coomans 1985; Palomares-Rius et al. 2008). Therefore, *Paralongidorus* diversity in China needs to be further investigated.

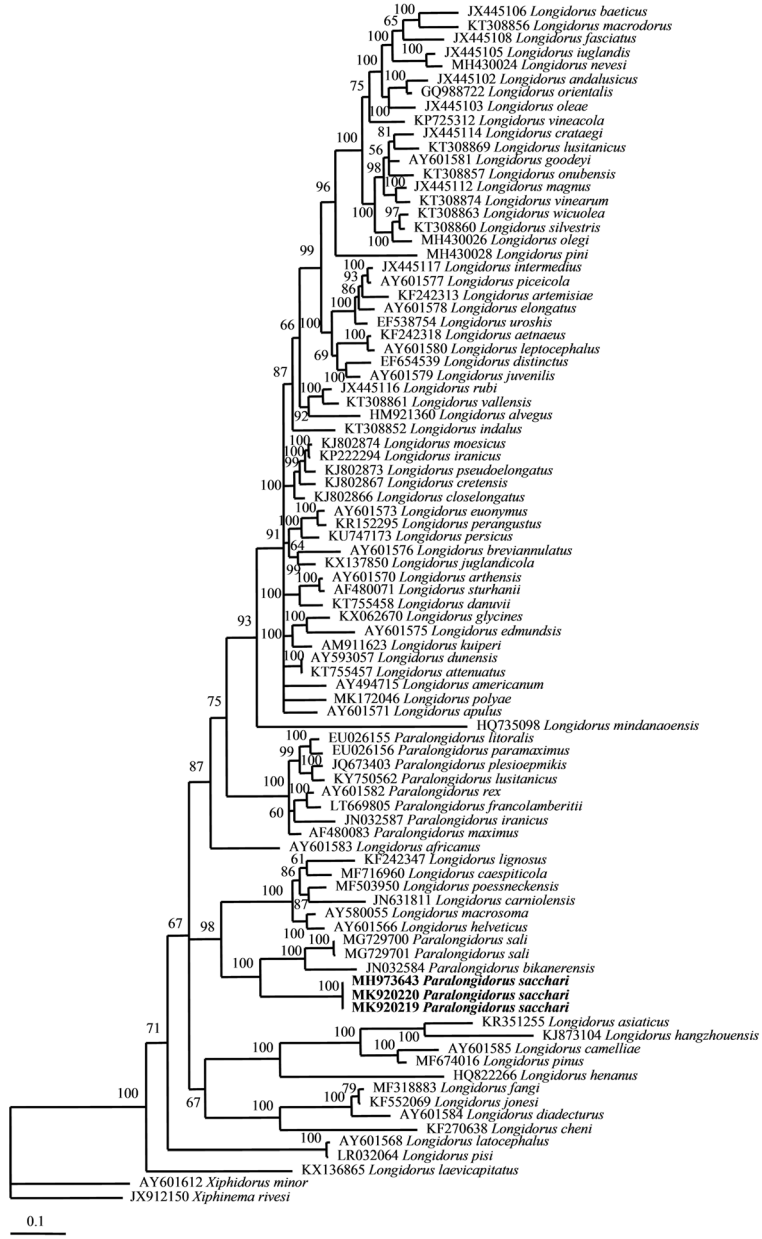


**Fig. 5** Bayesian consensus tree inferred from D2-D3 expansion segments of 28S rRNA gene of *Paralongidorus sacchari* population in China under GTR + I + G model (lnL = 15,454.2051; AIC = 30,928.4102; freqA = 0.2429; freqC = 0.2155; freqG = 0.2745; freqT = 0.2671; R(a) = 0.8533; R(b) = 2.4488; R(c) = 1.4415; R(d) = 0.6199; R(e) = 4.7947; R(f) = 1; Pinva = 0.3107; Shape = 0.8996). Newly obtained sequences are indicated in bold

Cuban royal palm is susceptible to red ring nematode, *Bursaphelenchus cocophilus* (Cobb, 1919) Baujard 1989 and burrowing nematode, *Radopholus similis* (Cobb, 1893) Thorne 1949 (Goodey et al. 1965; Chuo and Wouts 1977). No other plant nematodes were discovered from Cuban royal palm before. In this study, *P. sacchari* was isolated from Cuban royal palm, further evaluation on its pathogenicity and economic damage is needed.

Palms are good hosts of needle nematodes (*Paralongidorus* spp.). There are 6 species of needle nematodes having the ability to parasitize the plant in Arecaceae. Date palm (*Phoenix dactylifera*) is the host of *P. bikanerensis* (Pedram et al. 2012) and *P. georgiensis* (Tulaganov, 1937) Siddiqi 1964 (FAO 2009). Coconut palm (*Cocos nucifera*) is the host of *P. citris* (Khan et al. 1989) and *P. flexus* Khan et al., 1971 (Khan et al. 1971). Chile cocopalm (*Jubaea chilensis*) is

the host of *P. sacchari* (Roca and Rios 2006). Cuban royal palm (*Roystonea regia*) is anther Arecaceae host of *P. sacchari* as found in this study.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Human and animal rights** The article does not contain any studies with human or animal subjects performed by any of the authors.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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