**Iran, a new geographical distribution point for *Xiphinema santos* Lamberti,
Lemos, Agostinelli & D'Addabo 1993 (Nematoda: Longidoridae), a *X.*
americanum-group member**

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Abstract A population of *Xiphinema americanum*-group was recovered in association with stone fruit trees in Isfahan province, center of Iran. A reverse taxonomic approach based upon the large subunit ribosomal DNA (LSU rDNA D2-D3) and the mitochondrial cytochrome c oxidase subunit I (*COI* mtDNA) gene sequences in integration with morphological studies, revealed that the recovered population belongs to *Xiphinema santos*. The Iranian population was mainly characterized by 1240-1868 μ m long females with 60-84 μ m long odontostyle, a=37.2-51.9 and c=42.8-54.6. It is further characterized by a lip region having a shallow depression in junction with the body, presence of visible endosymbiont bacteria in ovaries under light microscope (LM), dorsally convex and ventrally slightly concave conical tail with blunt tip and three juvenile developmental stages. This population was identical with the type population in its morphology; however overlapped and extended morphometric data ranges as well as differences in some indexes were observed. Compared to a Spanish population of this species, the Iranian population had an identical morphology, similar morphometric data ranges and identical LSU and *COI* sequences. In LSU phylogeny, the relationship between the present and some previously sequenced isolates of the species and some isolates of three species *X. georgianum*, *X. laevistriatum* and *X. citricolum* was not resolved. In *COI* phylogeny, the clade of the Iranian and Spanish populations appeared as an independent lineage inside a major clade including several species. The comparison with other populations of the species was reported and discussed. A second species, *X. primum*, that is native to Iran was recovered from a new locality and characterized molecularly.

Keywords *COI* mtDNA, first report, LSU rDNA D2-D3, molecular, morphology, morphometrics, phylogeny, stone fruit trees, taxonomy, *Xiphinema primum*.

Introduction

The *Xiphinema americanum*-group currently includes 60 species (Mobasserri et al. 2019). The group is known with 10 representatives in Iran (Pedram 2018; Mobasserri et al. 2019). *Xiphinema santos* Lamberti et al. 1993 was originally described from Portugal in association with *Vitis* sp. (Lamberti et al. 1993) and was later reported from Portugal, Egypt and Spain in association with hop, pine, peach, grapevine and stone pine (Lamberti et al. 1993, 1994, 1996; Gutiérrez-Gutiérrez et al. 2012). In our recent study, a population of the species was recovered from Isfahan province in association with stone fruit trees and studied based upon morphological and molecular approaches. The species *X. primum* Mobasserri et al. 2019 that was originally described from Iran (Mobasserri et al. 2019) was also recovered from a new location in north of Iran and characterized molecularly.

Materials and methods

Sampling and morphological studies

Several soil samples were collected from a stone fruit garden with trees showing general weakness symptoms similar to those of nutrient deficiency in Isfahan province. At first, an infection with root-knot species was hypothesized and the roots were closely inspected. Furthermore, 15 soil samples were collected from Golestan province, north of Iran. Nematodes were extracted from soil samples using a tray method (Whitehead & Hemming 1965). A series of 20 and 60 mesh sieves (US standard mesh nos. equal to 850 and 250 μm sized openings, respectively) were used to extract tentative longidorid species. After extraction, the

specimens of interest were hand-picked under a Nikon SMZ1000 stereomicroscope, heat-killed by adding boiling 4% formaldehyde solution, and transferred to anhydrous glycerin according to De Grisse (1969). Permanent slides were prepared and morphological characters were studied using an Olympus BX51 light microscope equipped with differential interference contrast (DIC). The light microphotographs were taken using an Olympus DP72 digital camera attached to the microscope. Morphometric values were obtained using a drawing tube attached to Nikon E600 light microscope. The juvenile developmental stages were identified according to Robbins et al. (1996).

Molecular studies

For DNA extraction, two live *Xiphinema americanum*-group nematode specimens from the both populations recovered from the both provinces were picked out, washed in distilled water and photographed in temporary slides. Each selected nematode specimen was then transferred to a small drop of Tris-EDTA (TE) buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0, QIAGEN Inc., Valencia CA, USA) on a clean slide and squashed using a clean slide cover glass with the aid of a plastic material. Each DNA sample was taken from one individual. The suspension was collected by adding 20 µl of TE buffer. DNA samples were stored at –20°C until used as polymerase chain reaction (PCR) template. To amplify the near-full-length fragment of the LSU rDNA D2-D3 and partial *COI* mtDNA, several primer pairs were used in the PCR reactions. Primers used for the amplification of the D2–D3 expansion domains of the LSU rDNA were forward D2A (5'-ACAAGTACCGTGAGGGAAAGT-3') and reverse D3B (5'-TGCGAAGGAACCAGCTACTA-3') (Nunn, 1992) primers and in some cases, the reverse primer was replaced by the reverse primer KK28S-4 (5'-

GCGGTATTTGCTACTACCAYYAMG ATCTGC-3') (Kiontke et al. 2004). The used primers for amplification of the partial *COI* mtDNA were: forward primer JB3 (5'-TTTTTTGGGCATCCTGAGGTTTAT-3') and reverse primer JB4.5 (5'-TAAAGAAAGAACATAATGAAAATG-3') (Bowles et al. 1992), and forward primer COIF (5'-GATTTTTTTGGKCATCCWGARG-3') and reverse primer COIR (5'-CWACATAATAAGTATCATG-3') (Lazarova et al. 2006). The PCRs for amplification of the genomic and mitochondrial fragments were done according to Jahanshahi Afshar *et al.* (2019). The newly obtained sequences were submitted to the GenBank database under the accession numbers given in LSU and *COI* trees.

Phylogenetic analyses

The recently obtained LSU rDNA D2-D3 and *COI* mtDNA sequences were manually checked, edited and assembled. These sequences were compared with those of other relevant sequences available in the GenBank database using the BLAST homology search program. The BLAST search using one of the LSU sequences of the Isfahan province population revealed it has maximal identity with several isolates of *Xiphinema santos* (MN602712, MN602713, JQ990030, AY601587) and the BLAST search using its *COI* sequence revealed it has maximal identity with an isolate of *X. santos* (JQ990055). The BLAST search using the LSU sequence of the Golestan province population revealed it has 99.55-99.89% identity with several isolates of *X. primum* (MF372941-43, MF372947) and the BLAST search using its *COI* sequence revealed it has 93.07% identity with two isolates of *X. primum* (MK202795, MK202796). The DNA sequences for reconstructing the phylogenetic trees were selected according to recent studies (Archidona-Yuste et al. 2016; Mobasserri et al. 2019).

Multiple alignments of the LSU and *COI* datasets were performed using the Q-INS-i algorithm of online version of MAFFT version 7

1 <http://mafft.cbrc.jp/alignment/server/>) (Kato & Standley 2013). The Gblocks
2 program (version 0.91b) (Castresana 2000) with all three less stringent parameters,
3 a server tool at the Castresana Lab ([http://molevol.cmima.
4 csic.es/castresana/Gblocks_server.html](http://molevol.cmima.csic.es/castresana/Gblocks_server.html)), was used for post-editing of the alignment,
5 i.e., to eliminate poorly aligned regions or divergent positions. The best-fitting
6 substitution model for each of the two datasets was selected according to the Akaike
7 information criterion (AIC) by using PAUP*/MrModeltest.2 (Nylander 2004). A
8 general time reversible model, including among-site rate heterogeneity and estimates
9 of invariant sites (GTR+G+I) was selected and used for both LSU rDNA D2-D3 and
10 *COI* mtDNA datasets analyses. Bayesian analysis was performed using MrBayes
11 v3.1.2 (Ronquist & Huelsenbeck 2003) with a starting random tree and running the
12 chains for 5×10^6 generations for the both datasets. The Markov chains were sampled
13 every 100 generations for estimating the posterior probabilities of the phylogenetic
14 trees (Larget & Simon 1999) using the 50% majority rule. Of the converged runs,
15 25% were regarded as 'burn-in'. Convergence of model parameters and topology
16 were assessed based on average standard deviation of split frequencies and potential
17 scale reduction factor values. Adequacy of the posterior sample size was evaluated
18 using autocorrelation statistics as implemented in Tracer v.1.6 (Rambaut &
19 Drummond 2009). The output files of the phylogenetic programs used herein were
20 visualized using Dendroscope V.3.2.8 (Huson & Scornavacca 2012) and re-drawn
21 in CorelDRAW software version 17. The Bayesian posterior probability (BPP) value
22 exceeding 0.70 are given on appropriate clades.

24 **Results and description**

25 *Xiphinema santos* Lamberti, Lemos, Agostinelli & D'Addabo 1993

(Figures 1 & 2, Tables 1&2)

Female Body cylindrical, tapering gradually towards the extremities, ventrally curved, varying from an open to closed C, the posterior end more ventrally bent when heat-killed. Cuticle 2.0-3.5 μm thick along body, 1.5-2.0 μm thick in post labial region, 2-4 μm thick at mid-body, 3-4 μm thick just anterior to anus and 5-12 μm thick at tail tip. Lateral chord 10-16 μm wide at mid-body, occupying 27-38% of corresponding body diam. Lip region anteriorly rounded, having a shallow depression in junction with the body, slightly expanded, 11-12 μm wide and 4-5 μm high. Odontostyle 1.4-2.1 times longer than the odontophore, the latter with well-developed flanges. The guiding sheath 1-9 μm long. The pharyngeal basal bulb 55-76 μm long and 13-23 μm wide, occupying about 1/3 to 1/5 of the total pharynx length (Fig. 1C) with three pharyngeal nuclei, the larger dorsal gland nucleus (DN) located at 18.9-25.0% and the two smaller ventrosub lateral nuclei (S1N) located at 58-63% of pharyngeal bulb (location of glands' nuclei according to Loof and Coomans, 1972). Cardia rounded, 4-8 $\times$ 6-11 μm in size, intestine simple, prorrectum with great range in length (138-227 μm long). Reproductive system didelphic-amphidelphic with two seemingly equally developed branches, the anterior and posterior genital branch 108-186 and 111-233 μm long, respectively. Vulva a transverse slit, vagina 16-19 μm long with short *pars distalis vaginae* and well-developed *pars proximalis vaginae*. Prorrectum often indistinct. Rectum 13-22 μm long, or 0.6-1.1 times the anal body diameter. Tail short, conoid, weakly curved ventrally with rounded terminus, bearing two pairs of caudal pores.

Male Not found.

Juveniles Morphologically similar to females except for smaller body and narrower tails. Three juvenile developmental stages were detected. The scatter diagram separating juveniles and females is given in Fig. 2. All three juvenile

developmental stages were separated from each other using body length, length of replacement and functional odontostyle. In the first juvenile developmental stage, the tip of the replacement odontostyle is close to the base of the functional odontostyle, and in two rest stages, it is distantly located. All three juvenile stages have conical dorsally convex and ventrally concave tail.

Habitat and locality

The Iranian population of *X. santos* was recovered in association with stone fruit trees in Aran va Bidgol, Isfahan province (GPS coordinates are N 34.041459°, E 51.480842°, altitude: 918 masl).

Xiphinema primum Mobasseri, Hutchinson, Jahanshahi Afshar & Pedram, 2019

A population of this species was recovered in the present study from Golestan province (GPS coordinates are N 36°46.150', E 54°34.568') and sequenced for its LSU D2-D3 and *COI* mtDNA regions. The recovered population was in accordance with the type population and the newly generated sequences for this population are presented in corresponding trees. This finding shows the species could be more prevalent in the country than already assumed.

Molecular characterization and phylogenetic relationships

To determinate the phylogenetic affinities of the Iranian population of *Xiphinema santos* and the newly recovered population of *X. primum* with other species in the Longidoridae Thorne 1935 (and mostly with those species belonging to the *X.*

1 *americanum*-group), two separate LSU rDNA D2-D3 and *COI* mtDNA datasets
 2 were prepared for Bayesian inference (BI). For LSU phylogeny (Bayesian tree), a
 3 total number of 81 sequences, including the newly generated sequences and
 4 sequences of *Xiphinema non-americanum* group (*X. index* Thorne & Allen 1950
 5 and *X. diversicaudatum* (Micoletzky 1927) Thorne 1939), a Tylencholaimidae
 6 Filipjev 1934 member (*Tylencholaimus teres* Thorne 1939) and a Qudsianematidae
 7 Jairajpuri 1965 member (*Ecumenicus monohystera* (de Man 1880) Thorne 1974),
 8 as outgroups (accession numbers in Fig. 3) were selected. The LSU dataset was
 9 composed of 722 characters of which 308 were variable. The average nucleotide
 10 composition was: A: 23.5%, T: 18.7%, C: 25.8%, G: 32.0%. Figure 3 represents
 11 the Bayesian phylogenetic tree inferred using the above-mentioned dataset. In this
 12 tree, the two sequences of the Iranian isolate of *X. santos* (MN602712, MN602713)
 13 and some sequences from the previously sequenced isolates of the species and
 14 some isolates of three species *X. georgianum* Lamberti & Bleve-Zacheo 1979, *X.*
 15 *laevistriatum* Lamberti & Bleve-Zacheo 1979 and *X. citricolum* Lamberti & Bleve-
 16 Zacheo 1979 formed a highly supported clade (0.94 BPP). No interspecies
 17 cladogenesis was observed inside this clade. The newly generated sequence for *X.*
 18 *primum* also fell into the clade of the species.

19 A total number of 66 sequences, including the newly generated sequences, plus
 20 four sequences of *Xiphinema non-americanum* group spp. (*X. index*, *X.*
 21 *diversicaudatum* and *X. setariae* Luc 1958) as outgroups, were selected for *COI*
 22 mtDNA Bayesian phylogeny (accession numbers in Fig. 4). The *COI* dataset was
 23 composed of 391 characters of which 222 were variable. The average nucleotide
 24 composition was as follows: A: 25.4%, T: 37.1%, C: 15.1%, G: 22.4%. Figure 4
 25 represents the Bayesian phylogenetic tree inferred using this dataset. Based upon
 26 this tree, the newly generated sequence of the Iranian population of *X. santos*

(MN627267) formed a highly supported clade (0.99 BPP) with the Spanish isolate (JQ990055). All currently available *COI* sequences of *X. primum* formed a clade too.

Discussion

A relatively high density population (around 50 individuals/200 gr soil) of *X. santos* was recovered from the rhizosphere of stone fruit trees in a garden in Aran va Bidgol. Compared to the type population, the Iranian population has an identical morphology, but some differences in morphometric data ranges i.e. shorter body (1.6 (1.2-1.9) vs 1.8 (1.7-2.0) mm) and smaller a (44.3 (37.2-51.9) vs 54 (54-59)) were observed. For most other indexes, the ranges were overlapped; however, present study extended the morphometric data ranges for the species (Table 1). Compared to other populations reported from Portugal (Lamberti et al. 1994) and Egypt (Lamberti et al. 1996), again extension in indexes was observed (Table 1). Compared to the Spanish population, more similarity in some indexes like body and odontophore length and c ratio was observed. In summary, *X. santos* has a wide intraspecies ranges for body length of females (1.2-2.0 mm). Such a wide range for body length was also observed for the third juvenile developmental stage of the Egyptian population of the species (Lamberti et al. 1997). The wide range for body length has already been reported for some species like *Xiphinema* sp.1 (isolate CD47) by Orlando et al. (2016) (L=1193-2268 or 1.2-2.3 mm), *X. plesiopachtaicum* Archidona-Yuste et al. 2016 (L = 1.5-2.1 mm) and *X. parabrevicolle* Gutiérrez-Gutiérrez et al. 2012 (L=1.4-2.0 mm). The present study showed that *X. santos* also belongs to the group of species having a wide body length range. The species has three juvenile developmental stages (Lamberti et al.

1 1997) and the reported four juvenile developmental stages by Gutiérrez-Gutiérrez
2 et al. (2012) is amended herein.

3 In molecular phylogenetic analyses, similar to our previous study (Mobasser
4 et al. 2019), the relationships of *X. santos* with three species *X. georgianum*, *X.*
5 *laevistriatum* and *X. citricolum* was not resolved in LSU tree due to polytomy.
6 Although *X. citricolum* is shown to be the most closely related species to *X. santos*
7 in most LSU analyses (He et al. 2005; Gutiérrez-Gutiérrez et al. 2012; Lazorava et
8 al. 2016; Orlando et al. 2016; Mobasser et al. 2019), the effect of the
9 alignment/postediting methods on the resulted topology should not be ruled out.
10 The topology of the present LSU tree and dividing of the species into two
11 subclades I and II is similar to the resolved topology by Mobasser et al. (2019).

12 The *COI* mtDNA of *Xiphinema americanum*-group species has an acceptable
13 interspecies variation (Lazarova et al. 2016; Gutiérrez-Gutiérrez et al. 2012;
14 Orlando et al. 2016; Archidona-Yuste et al. 2016) and could yield better
15 cladogenesis in the corresponding tree. In our *COI* phylogeny, *X. santos* has
16 appeared as an independent lineage in a major clade including several other
17 species. The general topology and the subclades resolved in the present *COI* tree
18 are similar to the *COI* tree presented by Mobasser et al. (2019).

19 This is the first record of the species from Iran, corroborating its occurrence
20 out of the Mediterranean Basin, after its several reports from Portugal, North Egypt
21 and Spain (Lamberti et al. 1993, 1994, 1996; Gutiérrez-Gutiérrez et al. 2012).

23 Conclusion

24 In this study, *Xiphinema santos*, a member of *X. americanum*-group was reported
25 for the first time from Iran, extending its morphometric data ranges like body
26 length and the geographical distribution areas.

1

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5

6 **Compliance with ethical standards**

7 **Conflict of interest** All the authors certify that they do not have any conflict of
8 interest, the presented data are original and the presented data are not published,
9 and are not under evaluation for publication elsewhere, all prevailing local,
10 national and international regulations and conventions, and normal scientific
11 ethical practices, have been respected. They also certify that the final version of
12 this manuscript has been reviewed by all authors.

13

14 **Research involving human participants and/or animals** The soil samples
15 analyzed in this study were collected under the direct monitoring of the garden
16 owner or from the natural public regions that are not under protection against soil
17 samplings.

18

19 **Informed consent** The authors certify that they have accepted the principles of
20 ethical standards and that guidelines of professional conduct have been followed.
21 The funders had no role in designing of this study, collecting and analyzing of the
22 data, preparing the manuscript and are not involved in evaluating or publishing
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24

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1 methods for extracting small vermiform nematodes from soil. *Annals of Applied*
2 *Biology*, 55, 25–38.

14 Figure 1: Light microphotographs of Iranian population of *Xiphinema santos*
15 Lamberti et al. 1993. (A, B) Anterior region. (C) Pharyngeal bulb (D) Vulval
16 region. (E-H) Tail of the first, second and third stage juveniles and female,
17 respectively. (I) Ovary and endosymbiont bacteria. (All scale bars = 10 μ m).

19 Figure 2. Correlation of functional and replacement odontostyle to body length in
20 juvenile developmental stages and females of Iranian population of *Xiphinema*
21 *santos* Lamberti et al. 1993. The replacement odontostyle in each juvenile stage is
22 equal to the functional odontostyle in the next stage, and in J3, is equal to the
23 functional odontostyle length in females. (Ods. = Odontostyle).

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2 Figure 3. Bayesian 50% majority rule consensus tree inferred using LSU rDNA
3 D2-D3 sequences of Iranian population of *Xiphinema santos* Lamberti et al. 1993.
4 The Bayesian posterior probability values are given for the clades. The newly
5 generated sequences are in bold.

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7 Figure 4. Bayesian 50% majority rule consensus tree inferred using *COI* mtDNA
8 sequences of Iranian population of *Xiphinema santos* Lamberti et al. 1993. The
9 Bayesian posterior probability values are given for the clades. The newly generated
10 sequences are in bold.

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Table 1 Morphometric data for females of *Xiphinema santos* Lamberti et al. 1993. All measurements in μm and in the format: mean $\pm$ s.d. (range)

Origin	Iranian population	Lamberti et al. (1993) (Type population)	Lamberti et al. (1994) (Total range of three Portugal populations from Azores, Madeira and Braga)	Lamberti et al. (1996) (Egypt pop.)	Gutiérrez-Gutiérrez et al. (2012) (Spain pop.)
Characters	Females	Females	Females	Females	Females
n	22	20	15	22	9
L	1578.2 $\pm$ 134.0 (1240-1868)	1800 $\pm$ 800 (1700-2000)	1700-2000	1900 $\pm$ 100 (1700–2000)	1504 $\pm$ 75 (1466–1633)
a	44.3 $\pm$ 3.8 (37.2-51.9)	54.0 $\pm$ 2.3 (54-59)	41-49	51.0 $\pm$ 2.3 (47–54)	44.3 $\pm$ 2.1 (42.7–48.1)
b	5.7 $\pm$ 0.4 (4.9-6.3)	6.0 $\pm$ 0.4 (5.1-6.7)	5.7-7.5	6.5 $\pm$ 0.4 (5.7–7.1)	6.2 $\pm$ 0.9 (4.7–7.6)
c	48.4 $\pm$ 3.2 (42.8-54.6)	54.0 $\pm$ 3.1 (51-65)	51-62	53.0 $\pm$ 2.7 (48–57)	53.3 $\pm$ 6.2 (45.9–65.3)
c'	1.6 $\pm$ 0.1 (1.3-1.8)	1.7 $\pm$ 0.8 (1.5-1.8)	1.3-1.7	1.6 $\pm$ 0.1 (1.5–1.8)	1.3 $\pm$ 0.1 (1.0–1.4)
V%	52.3 $\pm$ 1.1 (50.1-54.0)	51.0 $\pm$ 1.7 (48-53)	47-53	52.0 $\pm$ 1.3 (49–53)	52.4 $\pm$ 1.1 (52–55)
Lip region width	11.3 $\pm$ 0.4 (11-12)	9-11	10-11	10-11	10.2 $\pm$ 0.7 (9.5–11.5)
Lip region height	4.2 $\pm$ 0.4 (4-5)	-	-	-	-
Odontostyle length	76.2 $\pm$ 6.6 (60-84)	83.0 $\pm$ 2.3 (79-88)	79-88	82.0 $\pm$ 1.8 (77–85)	78.9 $\pm$ 2.1 (76–82)
Odontophore length	42.7 $\pm$ 2.8 (36-47)	50.0 $\pm$ 1.4 (48-53)	46-52	47.0 $\pm$ 1.4 (44–49)	44.1 $\pm$ 2.3 (41–49)
Total stylet length	118.9 $\pm$ 8.1 (102-128)	-	-	-	-
Anterior end to guiding ring	65.0 $\pm$ 4.6 (56-73)	67.0 $\pm$ 1.8 (65-72)	60-70	68.04 $\pm$ 1.8 (63–71)	64.4 $\pm$ 1.8 (60–67)
Pharynx	279.8 $\pm$ 21.6 (238-319)	-	-	-	-
G1	9.2 $\pm$ 0.8 (7.8-10.5)	-	-	-	8.0 $\pm$ 0.9 (6.8–8.5)
G2	9 $\pm$ 2 (7.5-14.5)	-	-	-	7.5 $\pm$ 1.0 (6.4–8.9)
Lip region-vulva distance	827.3 $\pm$ 64.4 (666-981)	-	-	-	-
Body width at mid-body	36 $\pm$ 4 (29-44)	35.0 $\pm$ 2.2 (31-39)	35-45	37.0 $\pm$ 1.6 (35-40)	-
- at anus	20.8 $\pm$ 1.6 (19-24)	20.0 $\pm$ 1.2 (18-23)	19-23.5	22 $\pm$ 1 (20-24)	-
- at base of pharynx	31.1 $\pm$ 1.7 (28-33)	32.0 $\pm$ 1.3 (29-34)	32-42	33 $\pm$ 1 (31-35)	-
Tail	32.8 $\pm$ 2.5 (29-38)	34.0 $\pm$ 1.5 (32-37)	28-38	35.0 $\pm$ 1.2 (33–38)	28.5 $\pm$ 2.9 (25–34)

Table 2 Morphometric data of juveniles of Iranian population of *Xiphinema santos* Lamberti et al. 1993. All measurements are in μm and in the format: mean $\pm$ s.d. (range)

	J1	J2	J3
n	4	4	6
L	741.3 $\pm$ 31.8 (701-770)	901.8 $\pm$ 70.3 (818-990)	1168 $\pm$ 61.5 (1113-1286)
a	35.3 $\pm$ 1.6 (33.2-36.7)	40.2 $\pm$ 4.0 (35.6-45.1)	44.1 $\pm$ 2.1 (41.2-46.9)
b	4.4 $\pm$ 0.5 (3.8-4.8)	4.2 $\pm$ 0.3 (3.9-4.6)	4.7 $\pm$ 0.6 (4.1-5.5)
c	24.3 $\pm$ 1.1 (23.3-24.5)	26.5 $\pm$ 0.7 (25.6-27.3)	33.6 $\pm$ 1.8 (31.7-35.8)
c'	2.4 $\pm$ 0.2 (2.1-2.5)	2.3 $\pm$ 0.0 (2.3-2.4)	2.0 $\pm$ 0.1 (1.9-2.1)
Lip region width	8 $\pm$ 0.0 (8-8)	9.3 $\pm$ 1.0 (8-10)	10 $\pm$ 0 (10-10)
Odontostyle length	41.5 $\pm$ 0.6 (41-42)	49.5 $\pm$ 1.3 (48-51)	63.2 $\pm$ 3.1 (59-67)
Odontophore length	51.0 $\pm$ 0.8 (50/52)	83.0 $\pm$ 3.7 (79-87)	101 $\pm$ 3 (98-106)
Replacement odontostyle	49.3 $\pm$ 0.5 (49-50)	62.0 $\pm$ 2.9 (59-65)	76 $\pm$ 4.3 (72-84)
Anterior end to guiding ring	33.5 $\pm$ 1.0 (32-34)	41.8 $\pm$ 2.2 (39-44)	50.8 $\pm$ 2.0 (49-53)
Pharynx length	171.5 $\pm$ 13.3 (160-183)	213.8 $\pm$ 25.3 (189-238)	249.7 $\pm$ 28.1 (210-280)
Body width at mid-body	21.0 $\pm$ 0.8 (20-22)	22.5 $\pm$ 1.7 (20-24)	26.5 $\pm$ 1.5 (25-29)
- at anus	13 $\pm$ 1 (12-14)	14.58 $\pm$ 1.3 (13-16)	17.0 $\pm$ 0.6 (16-18)
Tail	30.7 $\pm$ 2.1 (29-33)	34.0 $\pm$ 2.6 (31-37)	34.8 $\pm$ 1.8 (32-37)