

Parapristionchus giblindavisi n. gen., n. sp. (Rhabditida: Diplogastridae) isolated from stag beetles (Coleoptera: Lucanidae) in Japan

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Summary – A new species of diplogastrid nematode, isolated in a previous survey of nematodes associated with stag beetles in Japan, is described as *Parapristionchus giblindavisi* n. gen., n. sp. *Parapristionchus* n. gen. differs from other diplogastrid genera chiefly by its stomatal morphology. Distinguishing the genus are the presence of a claw-like dorsal tooth in both the eurystomatous and stenostomatous forms and the division of the cheilostom into 12 plates lacking apical flaps. According to phylogenetic analysis of nine ribosomal protein gene sequences, *Parapristionchus* n. gen. shows deep divergence from other known genera. Molecular evidence strongly supports *P. giblindavisi* n. gen., n. sp. + *Pristionchus* spp. as monophyletic with respect to all other diplogastrids examined. Congruent with a clade of *P. giblindavisi* n. gen., n. sp. + *Pristionchus* spp. is the shared presence of a bifurcate P7 genital papilla. Discovery and description of a close sister group to *Pristionchus*, a model biological system, enables character polarisation in macroevolutionary studies of *Pristionchus* nematodes.

Keywords – *Dorcus rubrofemoratus*, molecular, morphology, morphometrics, new genus, new species, phylogeny, taxonomy.

The nematode family Diplogastridae Micoletzky, 1922 currently includes 30 genera (Sudhaus & Fürst von Lieven, 2003; Kanzaki *et al.*, 2009a; Mayer *et al.*, 2009; Fürst von Lieven *et al.*, 2011; Susoy & Herrmann, 2012), species of which are generally regarded to be insect-associated nematodes. Kanzaki *et al.* (2011) surveyed nematodes associated with stag beetles (Lucanidae) in Japan and reported seven diplogastrid species from eight species of stag beetles. Isolated nematodes included *Koerneria luziae* (Körner, 1954) Meyl, 1960, *K. lucani* (Körner, 1954) Meyl, 1960, *Pseudodiplogasteroides compositus* Körner, 1954, *Pseudodiplogasteroides* sp., *Rhabditoides* sp., *Pristionchus* cf. *pacificus*, and ‘*Pristionchus* sp.’ Among these seven species, the taxonomic affiliation of two species, *K. luziae* and ‘*Pristionchus* sp.’, were uncertain, as both were separated from their respective assigned

genera according to phylogenetic analysis of D2/D3 large subunit (LSU) rRNA and near full length small subunit (SSU) rRNA. ‘*Pristionchus* sp.’ was clearly differentiated from the *Pristionchus* clade by both morphological and molecular criteria, although it was closer to *Pristionchus* than any other genus included in that study.

The growing use of *Pristionchus* Kreis, 1932 as a model biological system (Sommer, 2009) highlights the need for resolved systematics of the genus. Studies of macroevolution, which place *P. pacificus* Sommer, Carta, Kim & Sternberg, 1996 as a focal point, require proper outgroups for polarising character transitions across *Pristionchus* species. Although ingroup relationships of the genus are increasingly resolved (Mayer *et al.*, 2007, 2009), studies have not agreed on placing an outgroup to the genus (Mayer *et al.*, 2007, 2009; van Megen *et al.*, 2009; Kion-

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tke & Fitch, 2010). Alleviating this disparity, the isolate reported as '*Pristionchus* sp.' was discovered as a taxon that breaks up the phylogenetic distance between *Pristionchus* and other diplogastrid genera (Kanzaki *et al.*, 2011). This species is described herein as *Parapristionchus giblindavisi* n. gen., n. sp. based on its apomorphic morphological characters and phylogenetic position as inferred from sequences of nine ribosomal protein genes.

Materials and methods

NEMATODE ISOLATION AND CULTIVATION

Parapristionchus giblindavisi n. gen., n. sp. was isolated from an adult of *Dorcus rubrofemoratus* Vollenhoven (Coleoptera: Lucanidae) collected in Iwazumi, Iwate Prefecture, Japan, in September 2010. The strain has been kept in laboratory culture on NGM agar plates seeded with *Escherichia coli* strain OP50, under the culture code and freezing voucher number RS5555. Detailed isolation conditions for the strain are described in Kanzaki *et al.* (2011).

MORPHOLOGICAL OBSERVATION AND PREPARATION OF TYPE MATERIAL

One- to 2-week-old cultures of *P. giblindavisi* n. gen., n. sp. were prepared for morphological observation. Light microscopic observations were conducted using live nematodes, which were handpicked from culture plates. To prepare type material, nematodes were isolated from cultures, rinsed in distilled water to remove bacteria, heat-killed at 65°C, fixed in 5% formalin, and processed through a glycerin and ethanol series using Seinhorst's method (see Hooper, 1986).

MOLECULAR CHARACTERISATION AND PHYLOGENY

In a previous study, all isolates other than that from Okinawa (see below) were confirmed to be the same species by their D2/D3 LSU and near full length SSU rRNA sequences (Kanzaki *et al.*, 2011), each of which was identical across isolates. Details about primers and sequences amplified are given therein. To confirm that the isolate collected from Okinawa belongs to the same species as the others, an 830 bp fragment of the SSU rRNA gene was extracted, amplified, and sequenced for the isolate. The SSU rRNA sequence was identical to that of the above isolates and is not discussed further herein. Information regarding primers, PCR conditions,

and sequence amplified for the latter isolate is given in Mayer *et al.* (2007).

For phylogenetic analysis, nine ribosomal protein genes were amplified and sequenced, generating a total alignment of 5996 coding nucleotides. Genes included in analysis were *rpl-2*, *rpl-10*, *rpl-14*, *rpl-23*, *rpl-29*, *rpl-35*, *rps-14*, *rps-27* and *rps-28*. Sequences for ribosomal protein genes have been deposited in GenBank under accession numbers JQ677914-JQ677920. All information regarding genes, primers, and PCR conditions is given in Mayer *et al.* (2009). Species selected for analysis include three undescribed species isolated from Japan (*Pristionchus* sp. 10, *Pristionchus* sp. 14, and *Pristionchus* sp. 15), which are the most basal known representatives of the genus *Pristionchus* and thus likely to be the *Pristionchus* species closest to *P. giblindavisi* n. gen., n. sp. Molecular sequence data for *Pristionchus* species were collected and reported by Mayer *et al.* (2007). Other species included represent other diplogastrid and outgroup genera for which homologous sequence data are already published (Mayer *et al.*, 2009). Isolation details for included *Pristionchus* species other than *P. giblindavisi* n. gen., n. sp. are given in Mayer *et al.* (2007) and Kanzaki *et al.* (2012). Isolation details for species of other genera are given in Mayer *et al.* (2009).

The concatenated dataset of ribosomal protein genes was aligned in ClustalX and then analysed in MEGA5.05 (Tamura *et al.*, 2011) after complete deletion of gapped or ambiguous positions. Substitution models were evaluated in MEGA5. The model with the lowest Bayesian Information Criterion (BIC) score Tamura-Nei93 + I + G, was chosen for analysis. Phylogenetic analysis was performed using the maximum likelihood (ML) criterion as implemented in MEGA5. The taxon *Rhabditoides inermis* (SB328) was specified as outgroup, as informed by more broadly representative phylogenetic analyses (van Meegen *et al.*, 2009). Node support was evaluated by 500 bootstrap pseudoreplicates.

Results

*Parapristionchus** n. gen.

DESCRIPTION

Diplogastridae. Body cylindrical, stout. Cuticle thick, with fine annulation and clear longitudinal striations. Lips

* Derived from the Greek *παρά* ('near') + *Pristionchus*, a closely related genus according to morphological and molecular evidence.

not clearly separated from each other or from rest of body. Six short and papilliform labial sensilla present in male and female, and four small, papilliform cephalic papillae present in male, as typical for diplogastrid nematodes. Stomatal dimorphism with stenostomatous (narrow mouth) and eury stomatous (wide mouth) forms occurring in both male and female. In both forms, cheilostom consisting of 12 well-cuticularised and conspicuous plates, and a thick, well-cuticularised gymnostom forming a short tube or ring. Stegostom bearing a conspicuous and movable dorsal, claw-like tooth in both forms. Stegostom bearing two bump-like (blunt) left subventral denticles and a small, short, and pointed right subventral denticle in stenostomatous form, and bearing a large, claw-like right subventral tooth and a row of left subventral denticles of varying numbers and size, *i.e.*, two large denticles to four small denticles, in eury stomatous form. Apodeme absent. Female gonads paired. Male with nine pairs of genital papillae, of which P7 (small ventral pair second from posterior) is distally split into two small papilla-like projections. Bursa or bursal flap absent.

TYPE AND ONLY SPECIES

Parapristionchus giblindavisi n. gen., n. sp.

RELATIONSHIPS

Parapristionchus n. gen. is distinguished from all other diplogastrids (Fürst von Lieven & Sudhaus, 2000; Sudhaus & Fürst von Lieven, 2003) by its cheilostomatal morphology, *i.e.*, 12 well-cuticularised thick plates in both male and female, and its molecular phylogenetic status inferred from near full length SSU (voucher sequence AB597234 deposited in GenBank by Kanzaki *et al.*, 2011). The new genus is morphologically and molecularly close to *Pristionchus* and, among known diplogastrid nematodes, is considered to be the sister group of the latter. A character shared between *Parapristionchus* n. gen. and *Pristionchus* is a distally bifurcate P7 genital papilla (see Kanzaki *et al.*, 2012). *Parapristionchus* n. gen. is distinguished from *Pristionchus* by its cheilostomatal structure in both the steno- and eury stomatous forms, having 12 well-cuticularised plates without clear flaps at the stomatal openings *vs* six per- and interradial plates with extended and rounded anterior parts that each form a flap-like apparatus covering the stomatal opening. Also distinguishing *Parapristionchus* n. gen. from *Pristionchus* is a small but claw-like dorsal tooth in the stenostomatous form *vs* a tooth with an inverted V shape in the steno-

stomatous form. According to phylogenetic analysis of SSU rRNA sequences, *Parapristionchus* n. gen. and *Pristionchus* form a well-supported monophyletic clade but are clearly distinguished from each other by deep branch lengths (Kanzaki *et al.*, 2011).

Parapristionchus n. gen. is morphologically close to *Micoletzkyia* Weingärtner, 1955 but is distinguished from the latter genus by its cheilostomatal structure in both the steno- and eury stomatous form, having 12 *vs* six plates and by the stegostom in both mouth forms, the postdental part of which in *Parapristionchus* n. gen. is not as deep as in *Micoletzkyia*. Further distinguishing *Parapristionchus* n. gen. from *Micoletzkyia* is the arrangement of male genital papillae, *viz.*, the close proximity of P1 and P2 papillae, which is characteristic of *Micoletzkyia* (Fürst von Lieven & Sudhaus, 2000; Sudhaus & Fürst von Lieven, 2003), is not observed in *Parapristionchus* n. gen.

MOLECULAR CHARACTERISATION AND PHYLOGENY

Analysis of the concatenated set of nine ribosomal protein genes revealed deep separation of *P. giblindavisi* n. gen., n. sp. from all other included extant genera (Fig. 1). Despite long branch lengths between *P. giblindavisi* n. gen., n. sp. and *Pristionchus* spp., these two genera form a monophyletic clade (97.2% bootstrap support, BS). The monophyly of *Pristionchus* spp. within this clade was also highly supported (100% BS). The present analysis failed to resolve relationships of *P. giblindavisi* n. gen., n. sp. + *Pristionchus* spp. to other groups. The next deepest node to this clade, shared with *Acrostichus* sp. + *Diplogasteriana schneideri*, did not have more than negligible support (40.6% BS, not shown).

***Parapristionchus giblindavisi** n. gen., n. sp.**
= *Pristionchus* sp. *apud* Kanzaki *et al.*, 2011
(Figs 2-6 and supplementary material)

MEASUREMENTS

See Table 1.

* We dedicate this species to Dr Robin M. Giblin-Davis in recognition of his extensive work on insect-associated nematodes.

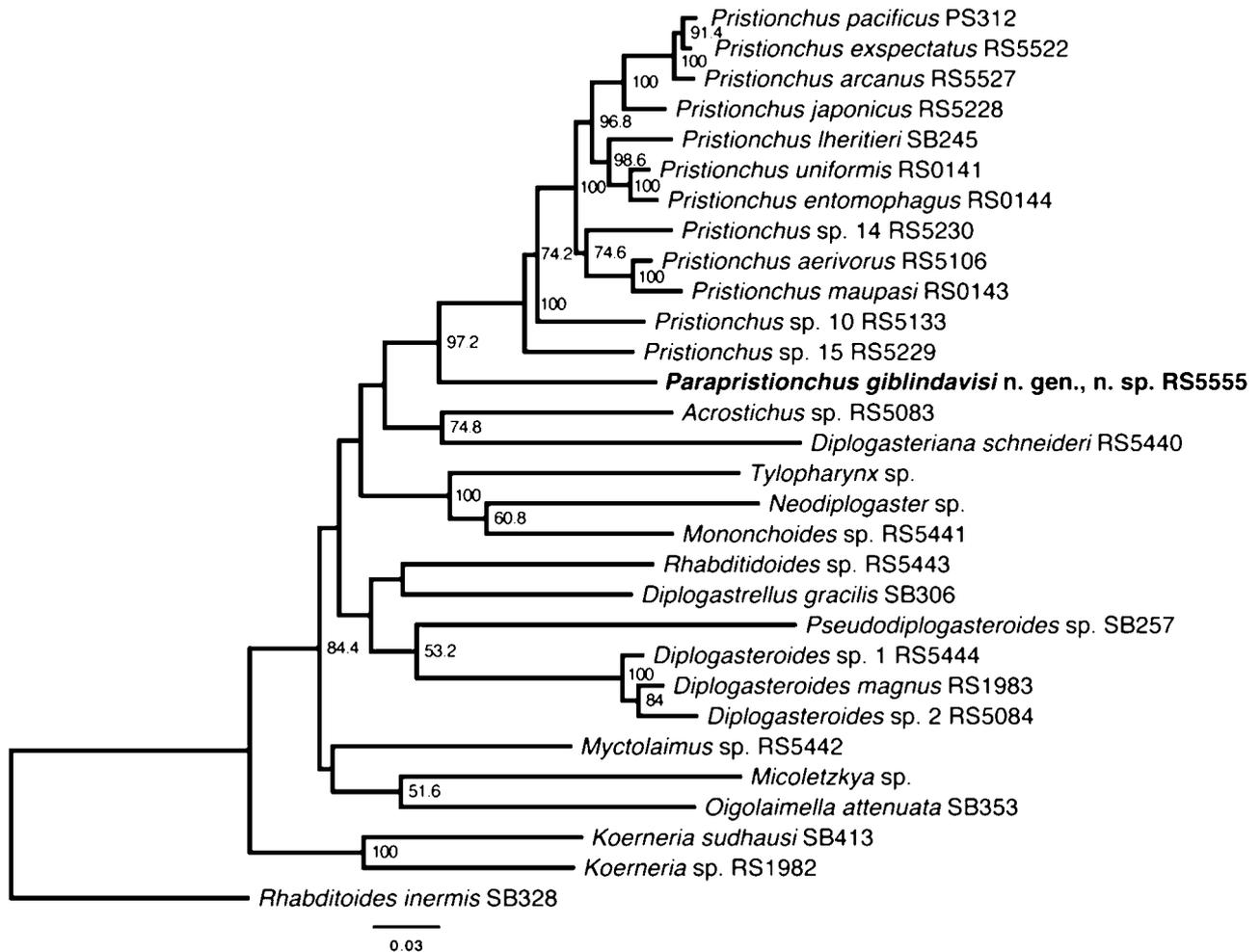


Fig. 1. Phylogenetic relationships of diplogastrid species, including *Parapristionchus giblindavisi* n. gen., n. sp., by maximum likelihood (ML). Analysis was performed using the Tamura-Nei model (Tamura & Nei, 1993) as implemented in MEGA5 (Tamura et al., 2011). There were a total of 3410 positions in the final dataset. The tree with the highest log likelihood ($-33\,137.8469$) is shown. The percentage of trees in which the associated taxa clustered together in 500 bootstrap pseudoreplicates is shown next to the nodes; percentages lower than 50% are not shown. Initial tree(s) for the heuristic search were obtained automatically as follows. When the number of common sites was <100 or less than one-fourth of the total number of sites, the maximum parsimony criterion was used; otherwise BIONJ with a MCL distance matrix was used. A discrete gamma distribution was used to model evolutionary rate differences among sites (five categories (+G, parameter = 0.5198)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 42.4501% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 30 concatenated nucleotide sequences. Codon positions included were 1st + 2nd + 3rd. All positions containing gaps and missing data were eliminated.

DESCRIPTION

Adults

Body cylindrical, stout. Cuticle thick, with fine annulation and clear longitudinal striations. Lateral field consisting of two lines, only weakly distinguishable from body striation. Head narrowly rounded, without appar-

ent lips, and with six short and papilliform labial sensilla. Four small, papilliform cephalic papillae present in males, as typical for diplogastrid nematodes. Amphidial apertures located at level of posterior end of cheilostomatal plates. Stomatal dimorphism present, with stenostomatous (narrow mouth) and eurystomatous (wide mouth) forms occurring in both male and female. De-

tailed stomatal morphology is described below. Dorsal pharyngeal gland clearly observed, penetrating dorsal tooth to gland opening. Anterior part of pharynx (= pro- and metacarpus) *ca* 1.6 times as long as posterior part (isthmus and basal bulb). Procorpus very muscular, stout, occupying half to two-thirds of corresponding body diam. Metacarpus very muscular, forming well-developed median bulb. Isthmus narrow, not muscular. Basal bulb glandular. Pharyngo-intestinal junction clearly observed, well developed. Nerve ring usually surrounding middle to posterior part of isthmus. Excretory pore not conspicuous, ventrally located at level of basal bulb to pharyngo-intestinal junction. Hemizonid not clearly observed. Deirids observed laterally, at the level of, or slightly posterior to pharyngo-intestinal junction. Post-deirids, presumed to be small gland openings, present and observed laterally, with positions inconsistent among individuals, numbering 5-8 for the male and 9-13 for the female.

Stenostomatous form

Cheilostom consisting of 12 cuticular plates. Incision between plates and vertical striation on the internal surface of the plates distinguishable by light microscopy. Anterior end of each plate reaching to stomatal opening, ending with squared or slightly irregular anterior edge. Gymnostom shorter than, or almost same depth as, cheilostom, with cuticular ring-like anterior part slightly overlapping with cheilostom medially. Dorsal gymnostomatal wall thickened compared with ventral side. Stegostom bearing conspicuous and movable dorsal claw-like tooth, two bump-like (blunt) left subventral denticles apparently projecting from a common cuticular plate, and a right subventral plate sometimes bearing small, short and pointed denticle. Dorsal tooth strongly sclerotised.

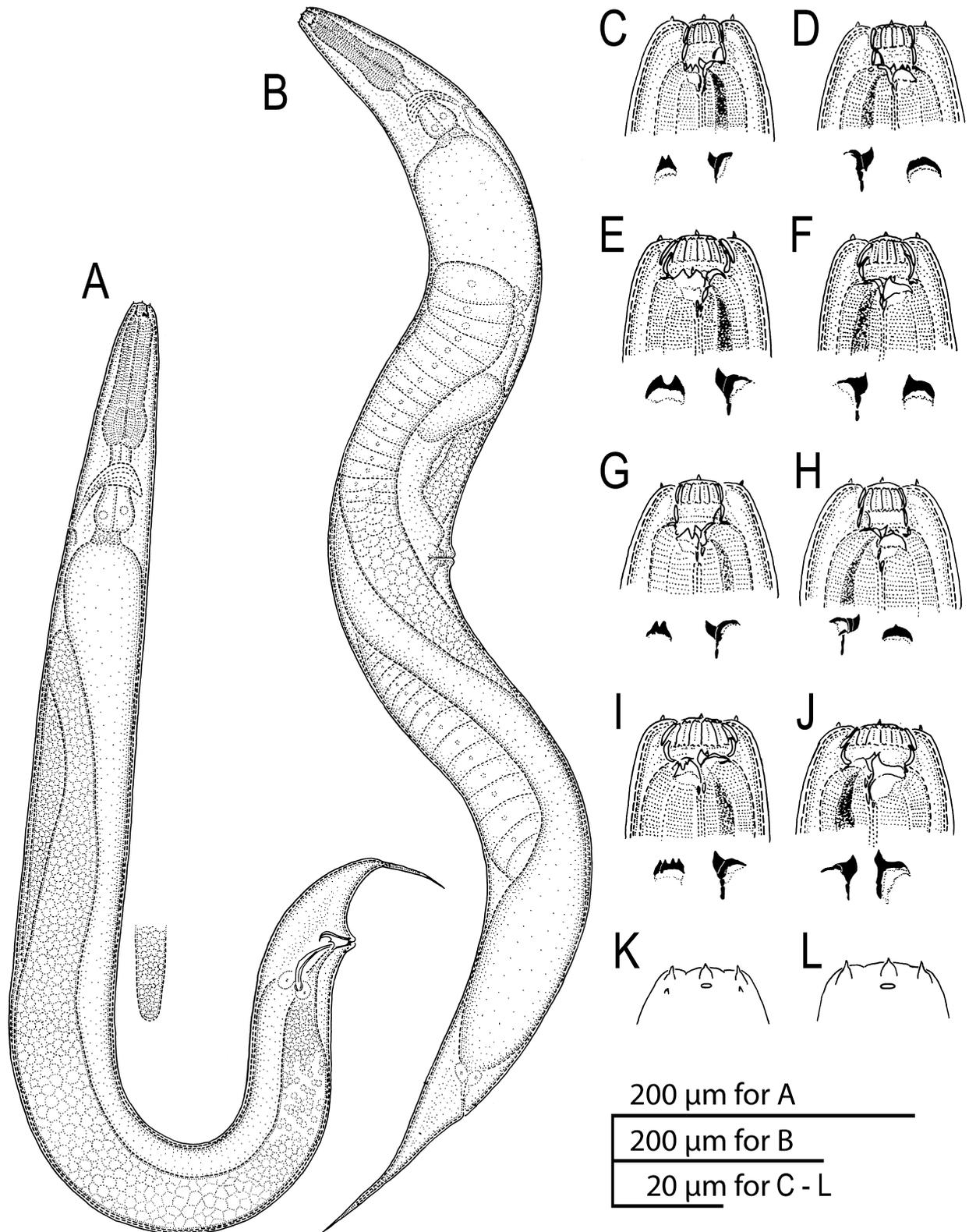
Eurystomatous form

Anterior part of buccal cavity (cheilo- and gymnostom) forming wide barrel-like or spherical shape, *i.e.*, with central part wider than stomatal opening and gymnostegostomatal margin. Cheilostom divided into 12 distinctive plates. Anterior end of each plate same as in stenostomatous form. Posterior end of each plate wider than anterior end, such that each plate forming a trapezoid. Gymnostom of same or greater depth as cheilostom, with thick cuticle, forming a short, ring-like tube and narrowing posteriorly. Anterior quarter to one-third of gymnostom overlapping medially with posterior part of cheilostomatal plates. Stegostom bearing a large, claw-like dorsal tooth, a large, claw-like right subventral tooth, and a row of left

subventral denticles with varying numbers and size, *i.e.*, two large denticles to four small denticles. Both dorsal and right subventral teeth movable. Left subventral denticles immovable.

Male

Ventrally arcuate, strongly ventrally curved at tail region when killed by heat. Testis single, ventrally located, anterior part reflexed to right side. Spermatogonia arranged in four to six rows in reflexed part, then well developed spermatocytes arranged as multiple (three to more than ten) rows in anterior two-thirds of main branch, then mature amoeboid spermatids arranged in multiple rows in remaining, proximal part of gonad. *Vas deferens* not clearly separated from other parts of gonad. Spicules paired, separate. Spicules smoothly curved in ventral view, adjacent to each other for distal third of their length, each smoothly tapering to pointed distal end. Spicule in lateral view smoothly ventrally arcuate, giving spicule about 100° curvature, rounded to roundish squared manubrium present at anterior end; lamina/calomus complex ventrally expanded at one-third length from anterior end, then smoothly tapering to pointed distal end. Gubernaculum conspicuous, about two-fifths of spicule in length, ear-like shape in lateral view, posterior half forming a tube-like process enveloping spicules. Dorsal side of gubernaculum well sclerotised. Distal end of gubernaculum possessing short pointed process on dorsal and on ventral side in lateral view. Tail conical, with spike 1.5-2.0 cloacal body diam. in length and having a filiform distal end. Cuticle thick around tail region, sometimes falsely appearing as a narrow leptoderan bursa in ventral view. Cloacal opening slit-like in ventral view. One small, ventral, single genital papilla on anterior cloacal lip. Nine pairs of genital papillae and a pair of phasmids present and arranged as (P1, P2d, P3, C, P4, P5d, Ph, (P6, P7, P8), P9d), where P3, cloacal slit and P4 are close to each other, and P3 is located more dorsally compared with P1 and P4. P6-P8 arranged linearly, P6 and P7 directed ventrad, P8 directed rather posteriad. Tip of P7 splitting into two small papilla-like projections. P9d located at level of or slightly posterior to P8. P1-P4 papillae of almost equal size, rather large and conspicuous, P5d slightly smaller than P1-P4, P6 and P7 very small, sometimes difficult to observe with light microscope, P8 and P9d small but larger than P6 and P7, *i.e.*, intermediate between P5d and P6/P7 in size. Bursa or bursal flap absent.



Female

Relaxed or slightly ventrally arcuate when killed by heat. Gonad didelphic, amphidelphic. Each genital system arranged from vulva/vagina as uterus, oviduct, and ovary. Anterior gonad on right of intestine, with uterus and oviduct extending ventrally and anteriorly on right of intestine and with a totally reflexed (= antidromous reflexion) ovary extending dorsally. Oviduct consisting of oval-shaped cells, with one or more single-celled to well developed egg(s) present in uterus and oviduct. Oviduct and ovary connected at gonad reflex by a crustaformerialike region of small, bubble-like spherical cells resembling a bunch of grapes. Oocytes mostly arranged in groups of four to eight or more, in rows in distal two-thirds to three-quarters of ovary and in single row in rest of ovary, one well-developed oocyte present at level just anterior to junction of ovary and oviduct, distal tips of each ovary reaching oviduct of opposite gonad branch. Anterior part of oviduct often filled with sperm, apparently serving as spermatheca. Eggs in single to multiple-cell stage or even further developed at posterior part of oviduct (= uterus). *Receptaculum seminis* not observed. Vaginal glands present but obscure, observed in ventral view. Vagina perpendicular to body surface, surrounded by sclerotised tissue. Vulva slightly protuberant in lateral view, pore-like in ventral view. Rectum *ca* 1 anal body diam. long, intestinal-rectal junction surrounded by well developed sphincter muscle. Three rectal glands, two ventral and one dorsal, present. Anus in form of dome-shaped slit, posterior anal lip slightly protuberant. Phasmid conspicuous, located *ca* 1-1.5 anal body diam. posterior to anus. Tail smoothly tapering or conical, with elongated distal part and filiform terminus.

Dauer juveniles

Occur in old culture plates. Body cylindrical, slender compared with adults. Cuticle smooth, covered with oily (lipophilic) substance sticking to plastic surfaces and

gathering bacteria. Lateral field forming narrow line but not easily observed. Lips fused to form dome-shaped or slightly flattened anterior end. Anterior end of stoma closed. Amphidial apertures clearly observed at level of middle part of indistinct cheilo/gymnostom. Stoma long, tube-like. Cheilostom and gymnostom vague, tube-like, occupying two-thirds of total stoma length. Stegostom clearly cuticularised, funnel-shaped, occupying one-third of total stoma length. Pharynx degenerate. Procorpus cylindrical, muscular. Metacarpus appearing as weakly developed median bulb. Metacarpus-isthmus junction not observed clearly. Isthmus long, slender. Terminal bulb elongated, oval, difficult to distinguish from isthmus. Pharyngo-intestinal junction visible, closed. Nerve ring surrounding anterior or middle part of isthmus. Deirid not clearly observed. Excretory pore visible, adjacent, or slightly anterior, to terminal bulb. Entire intestine occupied by lipid-like storage material. Genital *anlagen* occurring ventrally near mid-body. Rectum and anus visible. Phasmids clearly observed at 1.5 anal body diam. posterior to anus. Tail conical with pointed tip.

TYPE HOST (CARRIER) AND LOCALITY

The culture from which the type specimens were obtained was established from multiple emerging dauers isolated from the body of a single adult of *Dorcus rubrofemoratus* Vollenhoven (Coleoptera: Lucanidae) in Iwaizumi, Iwate Prefecture, Japan, by N. Kanzaki in September 2010.

TYPE MATERIAL

Holotype stenostomatous male (slide accession no. 30675), seven paratype stenostomatous males, two paratype eurystomatous males, six paratype stenostomatous females, and three paratype eurystomatous females (slide accession nos 30676-30693) deposited in the University of California Riverside Nematode Collection, Riverside,

Fig. 2. *Parapristionchus giblindavisi* n. gen., n. sp. A: *Stenostomatous* male, left lateral view; B: *Stenostomatous* female, right lateral view; C: Stomatal region of *stenostomatous* male, left lateral view; below (from left to right) are left subventral ridge and dorsal tooth; D: Stomatal region of *stenostomatous* male, right lateral view; below are dorsal tooth and right subventral plate without clear denticle; E: Stomatal region of *eurystomatous* male, left lateral view; below are left subventral ridge, and dorsal tooth; F: Stomatal region of *eurystomatous* male, right lateral view; below are dorsal tooth and right subventral tooth; G: Stomatal region of *stenostomatous* female, left lateral view; below are left subventral ridge and dorsal tooth; H: Stomatal region of *stenostomatous* female, right lateral view; below are dorsal tooth and right subventral denticle; I: Stomatal region of *eurystomatous* female, left lateral view; below are left subventral ridge and dorsal tooth; J: Stomatal region of *eurystomatous* female, right lateral view; below are dorsal tooth and right subventral tooth; K: Labial sensilla, cephalic papillae and amphid of *stenostomatous* male; L: Labial sensilla and amphid of *eurystomatous* female.

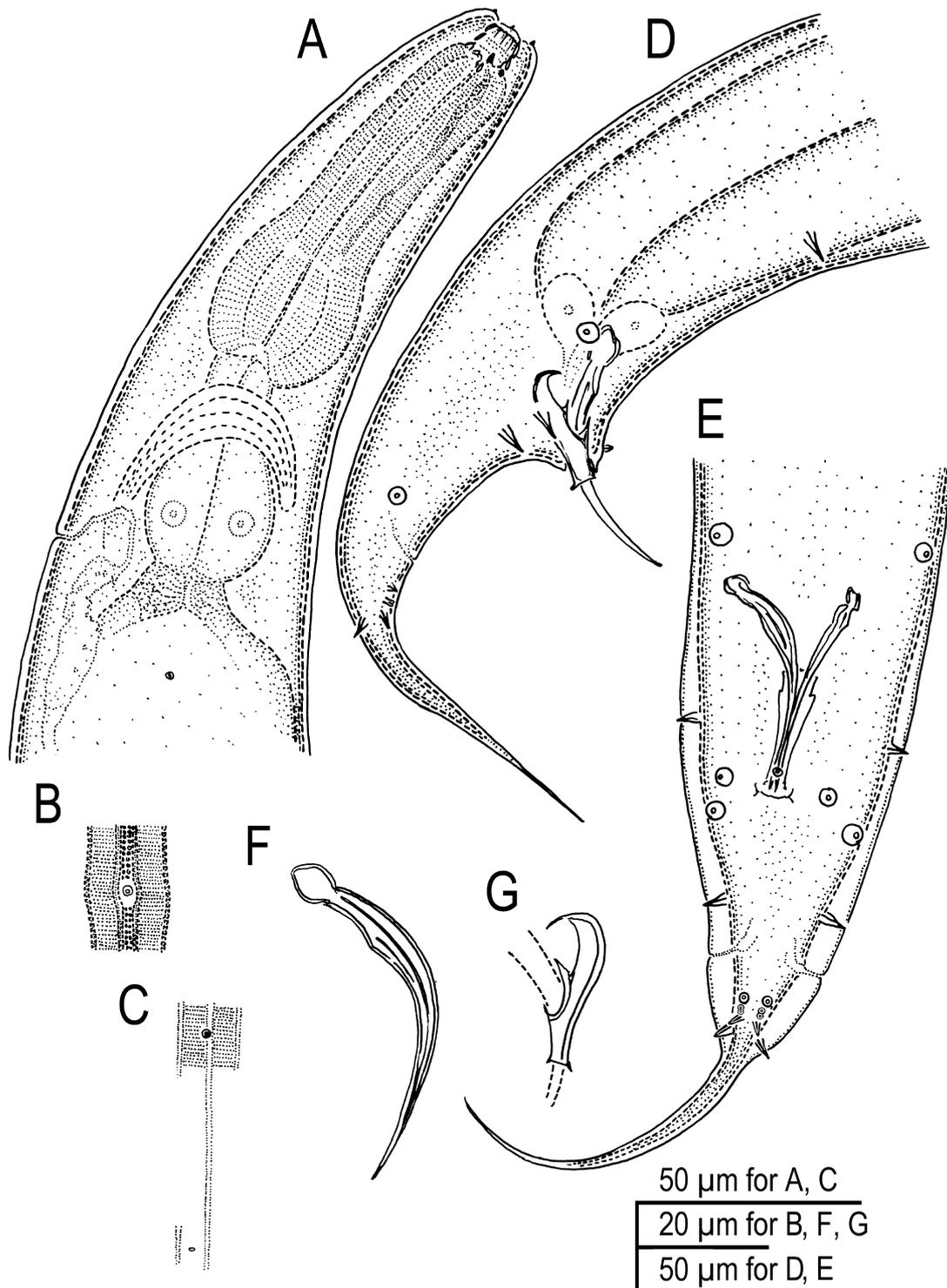


Fig. 3. Adult male of *Parapristionchus giblindavisi* n. gen., n. sp. A: Anterior end of stenostomatous form, left lateral view; B: Secretory-excretory pore, ventral view; C: Deirid (upper) and postdeirid (lower) openings, left lateral view; D: Male tail, right lateral view; E: Male tail, ventral view; F: Spicule; G: Gubernaculum.

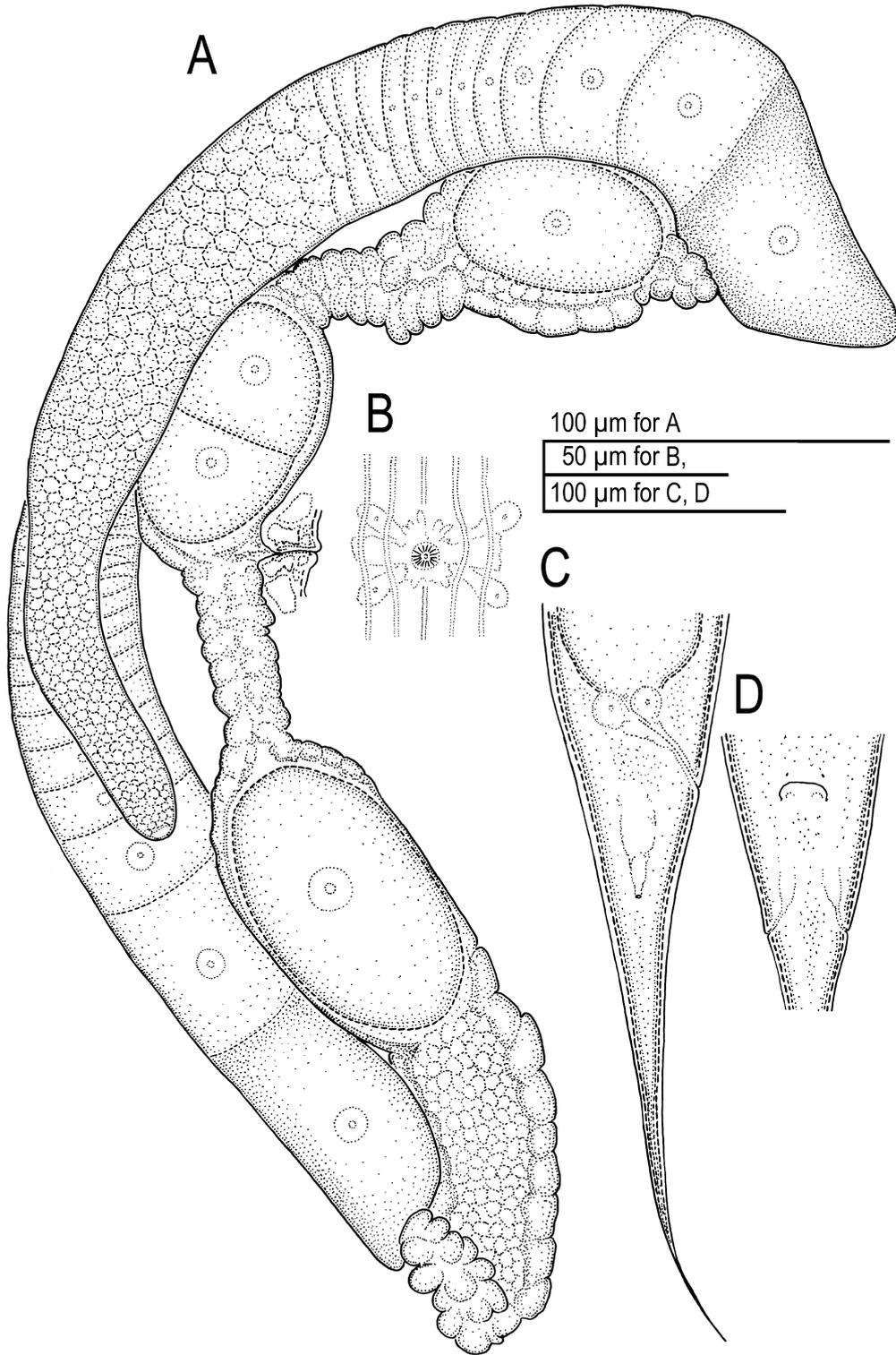


Fig. 4. Adult female of *Paraprisionchus giblindavisi* n. gen., n. sp. A: Reproductive tract, right lateral view; B: Vulval opening, ventral view; C: Tail region, right lateral view; D: Anus and phasmids, ventral view.

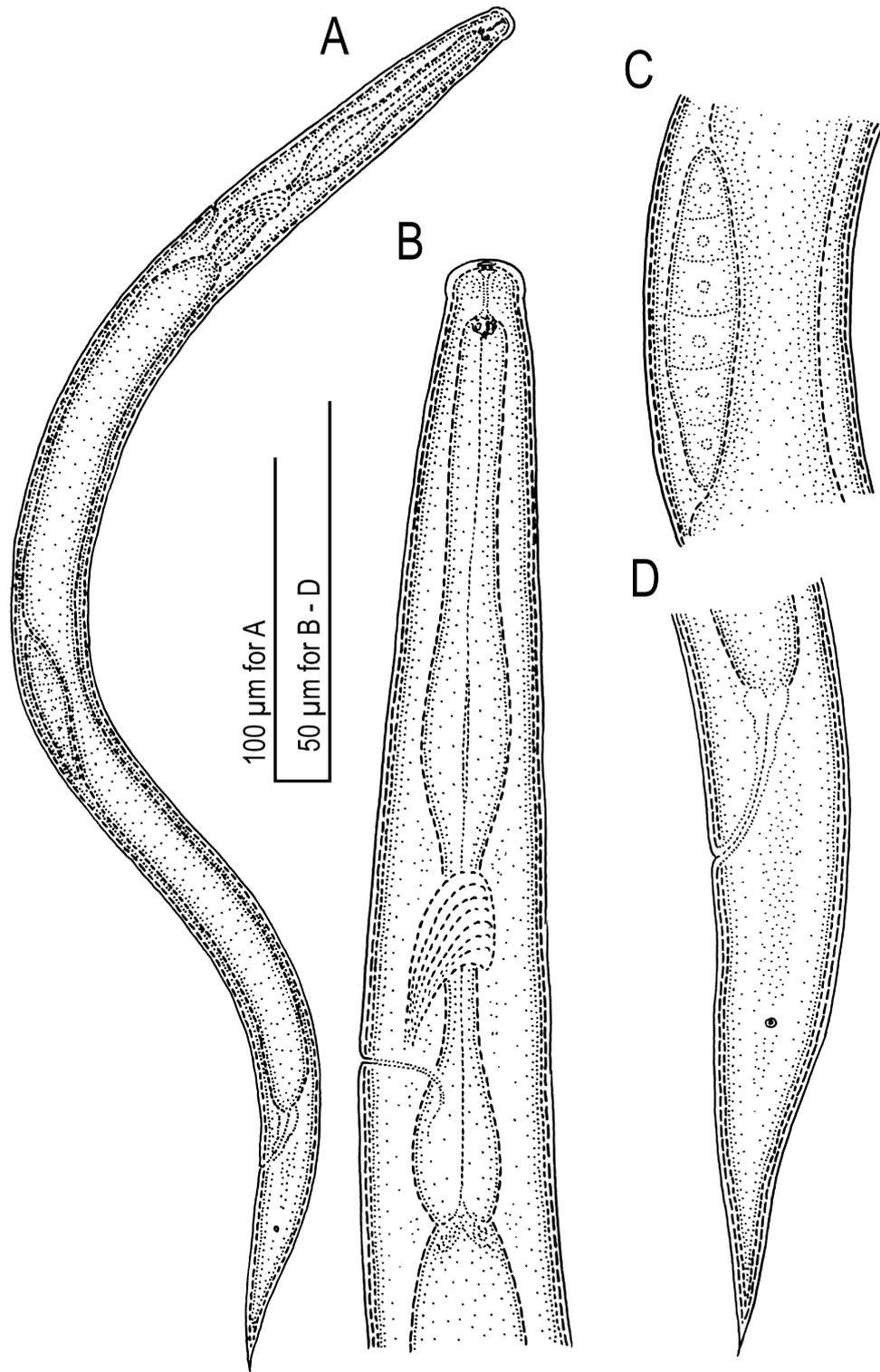


Fig. 5. *Dauer juvenile of Paraprisionchus giblindavisi n. gen., n. sp.* A: Entire specimen, left lateral view; B: Anterior end, left lateral view; C: Genital anlagen, left lateral view; D: Tail region, left lateral view.



Fig. 6. Nomarski micrographs of the stomatal region of *Parapristionchus giblindavisi* n. gen., n. sp. that highlight characters diagnostic of the new genus. A: Stenostomatous male, showing claw-like dorsal tooth; dorsal plane, right lateral view; B: Eurystomatous female, showing dorsal tooth and cheilostom without apical flaps (asterisk); dorsal plane, left lateral view; C: Eurystomatous female, showing left subventral ridge and cheilostomatal plates (arrows); left subventral plane, left lateral view. (Scale bars = 10 μ m.)

CA, USA. Five paratype stenostomatous males, four paratype stenostomatous females, and one paratype eurystomatous female (SMNK-Nema-T 0154-SMNK-Nema-T 0163) deposited in the Natural History Museum, Karlsruhe, Germany. Five paratype stenostomatous males, three paratype stenostomatous females, and two paratype eurystomatous females (SMNH Type-8280-SMNH Type-8289) deposited in the Swedish Natural History Museum, Stockholm, Sweden.

TYPE STRAIN CULTURE

Available as living cultures and frozen stocks under culture code RS5555 in the Department of Evolutionary Biology, Max Planck Institute for Developmental Biology, Tübingen, Germany, and can be provided to other researchers upon request.

DISTRIBUTION AND CARRIER RANGE

Besides its collection from the type host (carrier) and locality, the species was isolated from *Dorcus* spp. (Coleoptera: Lucanidae) obtained from five different localities in Japan: *D. rubrofemoratus* from Nishimeya, Aomori Prefecture, and from Iwaizumi, Iwate Prefecture; *D. striatipennis* from Tadami, Fukushima Prefecture and from Nakagawa, Tochigi Prefecture; and *D. titanus* from Aira, Kagoshima Prefecture (Kanzaki *et al.*, 2011). The species was additionally isolated from *D. titanus* on Iriomote Island, Okinawa Prefecture, Japan, by M. Herrmann in May 2011.

Discussion

Parapristionchus n. gen. is described herein as a monotypic genus characterised by stomatal morphology and by phylogenetic position as inferred from near full length SSU (Kanzaki *et al.*, 2011) and from nine, nuclear protein-coding genes (present study). Although it is close to *Pristionchus*, *Parapristionchus* n. gen. differs distinctly in key characters circumscribing the former genus. The division of the cheilostom into 12 per- and interradial plates is unique to the genus with respect to *Pristionchus* and other morphologically close genera, including *Micoletzkyia* and *Acrostichus* Rahm, 1928. Although this trait is found in more distant diplogastrid genera such as *Koerneria* Meyl, 1960, namely as described for males of *K. carinata* (Zullini, 1981) Fürst von Lieven & Sudhaus, 2000, current hypotheses of phylogeny (Mayer *et al.*, 2009; Kiontke & Fitch, 2010) suggest this character is convergent in the family. Supporting the probability of convergence is the labile nature of this trait in other diplogastrid genera, such as in *Fictor* Paramonov, 1952, which includes species (e.g., *F. vorax* Goodey, 1929) with 22 cheilostomatal plates, and in *Mononchoides* Rahm, 1928, in which the number of plates varies between sexes and even among individuals (Fürst von Lieven & Sudhaus, 2000). However, the constancy of cheilostomatal plate number within the closely related genus *Pristionchus* suggests the usefulness of this character for diagnosing *Parapristionchus* n. gen.

Also supporting *Parapristionchus* n. gen. as a genus distinct from *Pristionchus* is the presence of a claw-

Table 1. Morphometrics of stenostomatous male holotype (in glycerin) and male and female specimens of *Parapristionchus giblindavisi* n. gen., n. sp. (temporary water mounts). All measurements are in μm and in the form: mean \pm sd (range).

Character	Stenostomatous male		Eurystomatous male	Stenostomatous female	Eurystomatous female
	Holotype	Temporary water mounts	Temporary water mounts	Temporary water mounts	Temporary water mounts
n	–	10	10	10	10
L	1266	1226 \pm 58 (1160-1342)	1093 \pm 97 (994-1280)	1599 \pm 251 (1142-1959)	1150 \pm 162 (915-1474)
L'	1140	1096 \pm 53 (1033-1199)	957 \pm 91 (851-1128)	1387 \pm 218 (984-1687)	976 \pm 151 (767-1284)
a	15	17 \pm 0.8 (16-18)	18 \pm 1.6 (16-20)	15 \pm 1.5 (12-17)	15 \pm 1.6 (13-18)
b	8.3	7.7 \pm 0.2 (7.3-8.0)	6.5 \pm 0.4 (6.0-7.2)	9.4 \pm 0.7 (7.9-10.2)	6.7 \pm 0.6 (5.7-7.9)
c	10	9.4 \pm 0.5 (8.7-10.1)	8.1 \pm 0.7 (6.6-9.0)	7.5 \pm 0.3 (7.2-7.9)	6.6 \pm 0.6 (5.7-7.8)
c'	3.2	3.4 \pm 0.2 (2.8-3.6)	3.8 \pm 0.3 (3.5-4.2)	5.1 \pm 0.4 (4.5-5.9)	5.2 \pm 0.5 (4.3-6.1)
T or V	70	70 \pm 2.4 (66-74)	60 \pm 4.0 (54-66)	47 \pm 2.8 (39-49)	48 \pm 1.5 (44-49)
Max. body diam.	86	72 \pm 4.4 (66-79)	62 \pm 8.3 (51-76)	109 \pm 23 (89-158)	79 \pm 16.7 (60-115)
Stoma (cheilo- + gymnostom)	6.9	8.0 \pm 0.7 (7.1-9.5)	6.3 \pm 0.6 (5.4-7.1)	9.4 \pm 0.7 (8.6-10)	7.1 \pm 1.0 (5.4-8.5)
Neck length (head to base of pharynx)	159	159 \pm 4.9 (151-167)	167 \pm 8.4 (152-180)	171 \pm 26 (144-218)	171 \pm 8.8 (160-186)
Anterior pharynx (pro- + metacarpus)	89	85 \pm 3.1 (81-92)	98 \pm 4.3 (90-104)	93 \pm 13 (79-119)	101 \pm 5.7 (93-109)
Posterior pharynx (isthmus + basal bulb)	63	66 \pm 3.6 (62-74)	69 \pm 4.5 (62-77)	72 \pm 13 (59-89)	70 \pm 4.1 (65-77)
Post./ant. pharynx ratio	71	78 \pm 4.9 (68-87)	71 \pm 2.5 (66-75)	76 \pm 6.9 (69-91)	69 \pm 3.4 (63-74)
Excretory pore from ant. end	161	167 \pm 6.2 (159-176)	157 \pm 19 (133-186)	181 \pm 25 (139-222)	141 \pm 17 (109-160)
Testis length	887	853 \pm 39 (814-934)	656 \pm 94 (536-812)	–	–
Ant. female gonad (with flexure)	–	–	–	822 \pm 103 (604-964)	544 \pm 105 (414-740)
Post. female gonad (with flexure)	–	–	–	846 \pm 111 (616-1013)	530 \pm 113 (380-730)
Vulva to anus distace	–	–	–	640 \pm 89 (539-772)	426 \pm 71 (319-564)
Cloacal or anal body diam.	39	39 \pm 2.4 (35-43)	35 \pm 2.5 (32-41)	42 \pm 7.3 (34-54)	33 \pm 4.2 (29-44)
Tail length	126	130 \pm 8.5 (116-143)	136 \pm 16 (111-152)	213 \pm 34 (158-272)	174 \pm 15 (148-190)
Spicule length (arc)	62	60 \pm 3.0 (54-64)	57 \pm 3.3 (51-62)	–	–
Spicule length (chord)	50	51 \pm 2.2 (49-55)	48 \pm 3.3 (43-53)	–	–
Gubernaculum length	24	23 \pm 1.6 (21-26)	23 \pm 1.4 (20-24)	–	–

like dorsal tooth in the stenostomatous form. A qualitatively different dorsal tooth in the stenostomatous form is considered characteristic of *Pristionchus* (Sudhaus & Fürst von Lieven, 2003). Despite its variability in size, the stenostomatous dorsal tooth is remarkably consistent in form, namely as an inverted V, in described and undescribed species across the genus (E.J.R. & N.K., pers. obs.). Greater similarity of the dorsal tooth between forms, notwithstanding differences in tooth size, in *Parapristionchus* n. gen., *Acrostichus* and *Koerneria* suggest this character to be synapomorphic for *Pristionchus* spp. and therefore separating *Pristionchus* from other genera.

Genital papilla characters can be useful for distinguishing otherwise identical species of *Pristionchus* (Kanzaki *et al.*, 2012) but are apparently also informative for deeper relationships. Congruent with the monophyly of a clade of *Parapristionchus* n. gen. + *Pristionchus* spp. is the presence of a distally bifurcate P7 genital papilla in both taxa. This character, previously shown in SEM and drawings of *Pristionchus lheritieri* (Maupas, 1919) Paramonov, 1952 (Kiontke & Sudhaus, 2000), was found in several *Pristionchus* spp. (Kanzaki *et al.*, 2012) representing all lineages of the genus (Mayer *et al.*, 2007, 2009). In contrast, neither *Acrostichus* spp. (Kanzaki *et al.*, 2009b, 2010a, b) nor several undescribed *Micoletzkyia* spp. (N.K., pers. obs.) show this character. Interestingly, *Diplogasteroides nasuensis* Takaki, 1941 also shows a P7 papilla with a complex terminus that splits into three small tips arranged in a row (Kiontke *et al.*, 2001). Moreover, *Koerneria* spp. have non-simple termini for papillae P6-P8 (N.K., M.H., E.J.R., unpubl.; R. M. Giblin-Davis, pers. commun.). The phylogenetic separation of either *Diplogasteroides* spp. or *Koerneria* spp. from *Pristionchus* + *Parapristionchus* n. gen. (Mayer *et al.*, 2009) makes it possible that a “complex P7 terminus” is convergent. SEM-based investigation of the P7 papilla across Diplogastridae is needed to reconstruct detailed transformations of this apparently plastic character. At present, a bifurcate terminus is considered to be unique to *Parapristionchus* n. gen. and *Pristionchus*, although more taxon sampling will test whether this character state is truly synapomorphic.

Molecular phylogenetic evidence has been used both to discover and test the status of *Parapristionchus* n. gen. Analysis of SSU rRNA of *P. giblindavisi* n. gen., n. sp., as well as several species of *Pristionchus* and other diplogastrid genera, initially revealed deep molecular divergence where defining morphological characters were temporarily unknown (Kanzaki *et al.*, 2011). Detailed morphological observation and increased molecular sequence

data have now confirmed its separation and uniqueness. Deeper relationships of Diplogastridae were unresolved in the present study, but relative branch lengths show the extensive molecular divergence of *Parapristionchus* n. gen. from other genera, which is comparable to the distance separating other close genera. Moreover, among all taxa included in the study by Kanzaki *et al.* (2011), the number of predicted SSU rRNA substitutions separating *P. giblindavisi* n. gen., n. sp. from *Pristionchus* spp. was greater than that separating any other two sister genera in the family. Beyond this simple comparison, we do not recognise objective criteria for assigning supraspecific names based on sequence divergence alone, although we agree it is crucial to consult molecular phylogeny in revising higher taxa (Sudhaus, 2011).

It has been argued that monotypic genera should be avoided based on the inability to generalise across multiple reference points (Sudhaus & Fürst von Lieven, 2003). We contend that in a cladistic paradigm the error of paraphyly is more severe than that of increasing the number of classifiers, given that new or resurrected names represent monophyletic taxa. Because of the distinctiveness of *Parapristionchus* n. gen. based on multiple lines of evidence, we erect it to a new, monotypic genus. In a similar approach, Kanzaki *et al.* (2009a) erected the monotypic diplogastrid genus *Teratodiplogaster* based on clear morphological and molecular separation from other genera, since which time several new species have been discovered by additional survey work (Kanzaki & Giblin-Davis, unpubl.). The ongoing discovery of species in *Teratodiplogaster* and other insect-associated diplogastrid genera, including *Pristionchus* (Herrmann *et al.*, 2007, 2010), *Micoletzkyia* (Susoy *et al.*, unpubl.) and *Acrostichus* (Kanzaki *et al.*, 2009b, 2010a, b), lends credence to the possibility of expansion of *Parapristionchus* n. gen. upon further sampling of lucanids and other insects associated with similar sap or flux habitats.

Description of a closer outgroup to *Pristionchus* spp. has implications for studies of evolution within *Pristionchus*, which has become a model with advances in research on *P. pacificus* (Sommer, 2009). A comparative approach among close species enables testing hypotheses of macroevolution, for example of hermaphroditism and sex strategies (Mayer *et al.*, 2009; Kanzaki *et al.*, 2012), nematode-insect interactions (Hong & Sommer, 2006), and biogeography (Herrmann *et al.*, 2006; Mayer *et al.*, 2009; Morgan *et al.*, 2012). Considering the role of stoma morphology in describing *Parapristionchus* n. gen., the evolution of complex feeding structures is of

particular interest. The presence of a discrete dimorphism in *Pristionchus* nematodes enables studies of mechanisms responsible for complex (eurystomatous) or relatively reduced (stenostomatous) forms during development (Bento *et al.*, 2010). In principle, developmental studies can be extended to include multiple species to infer the role of the plasticity in morphological change. To support such studies, outgroup analysis can now more accurately polarise transition series of mouthpart homologies. The evolution of variability in the plasticity itself is likewise better understood by inference of ancestral states. Combined with detailed anatomical information (Baldwin *et al.*, 1997; Bumbarger *et al.*, unpubl.) and the capabilities of genetics studies, *Pristionchus* spp. and outgroup *Parapristionchus* n. gen. provide an unparalleled opportunity for studying the evolution of novel form in nematodes.

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Supplementary material

Video S1. Through-focal video of the anterior region of a stenostomatous male of *Parapristionchus giblindavisi* n. gen., n. sp.

Video S2. Through-focal video of the anterior region of a euryostomatous female of *Parapristionchus giblindavisi* n. gen., n. sp.

The videos can be accessed via <http://media.brill.nl/nemy/14/8/>.