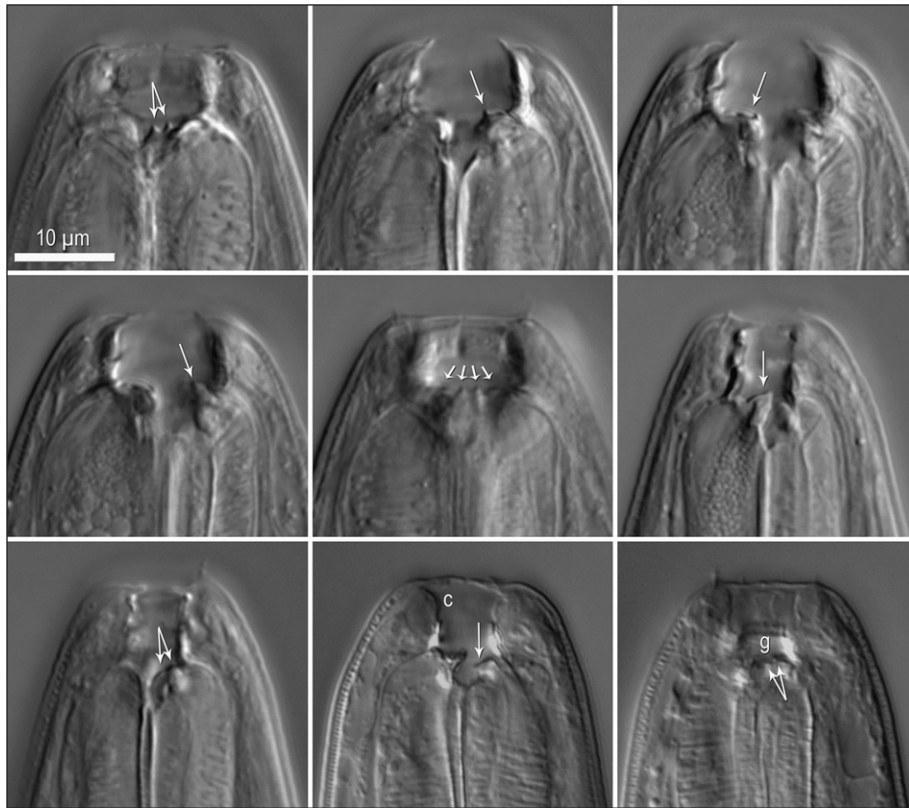


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Buccal cavity of eury- and stenostomatous *Pristionchus fissidentatus* n. sp.  
and of *Pristionchus elegans* n. sp.

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## Two New Species of *Pristionchus* (Rhabditida: Diplogastridae): *P. fissidentatus* n. sp. from Nepal and La Réunion Island and *P. elegans* n. sp. from Japan

NATSUMI KANZAKI,<sup>1,†</sup> ERIK J. RAGSDALE,<sup>2,†</sup> MATTHIAS HERRMANN,<sup>2</sup> RALF J. SOMMER<sup>2</sup>

**Abstract:** *Pristionchus fissidentatus* n. sp., isolated from soil in Nepal, and *P. elegans* n. sp., isolated from *Phelotrupes auratus* (Coleoptera: Scarabaeidae) in Japan, are described. The two new species are recognized as basal within the genus and thus occupy an important position for macroevolutionary studies that center on the model *P. pacificus*. *Pristionchus fissidentatus* n. sp. is distinguished by its unique stegostomatal morphology: in the stenostomatous form, the right subventral ridge has three prominent cusps and the left subventral sector has, in addition to a plate with two cusps, a prominent denticle slightly left of ventral; in the eury stomatous form, the right subventral stegostomatal sector shows both a tooth and a ridge with several cusps. Diagnostic of *P. elegans* n. sp. is the structure of the stenostomatous cheilostom, which bulges medially and is underlain by a large vacuolated ring. No eury stomatous form has been observed in *P. elegans* n. sp. Reproductive modes of *P. fissidentatus* n. sp. and *P. elegans* n. sp. are hermaphroditic and gonochoristic, respectively. The additional isolation of *P. fissidentatus* n. sp. from soil and two species of scarab beetle on La Réunion Island in the Indian Ocean suggests a broad geographic range for this species.

**Key words:** androdioecy, hermaphrodite, insects, morphology, morphometrics, new species, phylogeny, taxonomy.

The genus *Pristionchus* Kreis, 1932 is an increasingly well studied model that combines the disciplines of genetics, developmental biology, ecology, population genetics, and macroevolutionary studies (Sommer, 2009). To expand a system that centers on the species *Pristionchus pacificus* Sommer, Carta, Kim, and Sternberg, 1996, recent efforts have pushed for discovery of new species in the genus. Because *Pristionchus* species readily associate with beetles (Völck, 1950; Fedorko and Stanuszek, 1971; Herrmann et al., 2006a, 2006b, 2007, 2010; Kanzaki et al., 2011), targeting beetles and other potential carriers has seen reliable recovery of new *Pristionchus* isolates (Herrmann et al., 2006a, 2006b, 2010).

In their review of the family Diplogastridae Micoletzky, 1922, Sudhaus and Fürst von Lieven (2003) recognized 27 valid species of *Pristionchus*. Since that revision, Herrmann et al. (2006b) reported four new species isolated from scarab beetles in North America. On biological and molecular grounds, they described these species as *P. americanus* Herrmann, Mayer, and Sommer, 2006, *P. marianneae* Herrmann, Mayer, and Sommer, 2006, *P. pauli* Herrmann, Mayer, and Sommer, 2006, and *P. pseudaeivorus* Herrmann, Mayer, and Sommer, 2006. More recently described species include several from Japan that form a species complex with *P. pacificus*, namely *P. japonicus* Kanzaki, Ragsdale, Herrmann, Mayer, and Sommer, 2012 from a decaying earthworm, *P. arcanus* Kanzaki, Ragsdale, Herrmann, Mayer, and Sommer, 2012 from termites, and the putative sister species of *P. pacificus*, *P. exspectatus* Kanzaki, Ragsdale, Herrmann, Mayer, and Sommer, 2012, from stag beetles (Kanzaki et al., 2012a). In an ongoing effort to find and report new

*Pristionchus* species, we describe *P. fissidentatus* n. sp. and *P. elegans* n. sp., both in association with scarab beetles.

The importance of new species for comparative biology, which is a general aim of research centering on *Pristionchus* (Sommer, 2009), derives from their phylogenetic context. The recent discovery of a close outgroup to *Pristionchus*, *Parapristionchus gibbindavisi* Kanzaki, Ragsdale, Herrmann, Mayer, Tanaka, and Sommer, 2012, will enable better polarization of characters in macroevolutionary hypotheses (Kanzaki et al., 2012b). Additionally, denser taxon sampling between nodes of interest can improve accuracy of ancestral state reconstruction by reducing the phylogenetic distances between predicted changes. Published phylogenetic analyses (Mayer et al., 2007, 2009; Kanzaki et al., 2012a, 2012b) have identified *P. fissidentatus* n. sp. (called *Pristionchus* sp. 10 in those studies) and *P. elegans* n. sp. (called *Pristionchus* sp. 15) as taxa that break up the branches between outgroup taxa and the clade including all other sequenced *Pristionchus* species. Of the two species, *P. elegans* n. sp. is the most basal known species considered to be a congener, and *P. fissidentatus* n. sp., the next most basal (Mayer et al., 2007, 2009; Kanzaki et al., 2012a, 2012b). Detailed morphological characterization of these new species will therefore be useful for comparative studies across the genus.

### MATERIALS AND METHODS

**Nematode isolation and cultivation:** *Pristionchus fissidentatus* n. sp. was isolated from soil collected in Tatopani, Annapurna area, Myagdi, Nepal. Three additional isolates of *P. fissidentatus* n. sp. were collected on La Réunion Island: one from soil during one collection trip and two from scarab beetles, namely from *Adoretus Dejean* sp. and *Oryctes borbonicus* Dechambre, during a second trip. *Pristionchus elegans* n. sp. was isolated from an adult of *Phelotrupes auratus* (Motschulsky) (Coleoptera: Scarabaeidae) collected in a forest near Kutsuki,

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<sup>1</sup>Forest Pathology Laboratory, Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687 Japan.

<sup>2</sup>Max Planck Institute for Developmental Biology, Department of Evolutionary Biology, Spemannstraße 37, Tübingen, Germany.

<sup>†</sup>These authors contributed equally to this work.

E-mail: ralf.sommer@tuebingen.mpg.de

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Takashima-shi, Shiga Prefecture, Japan. For both species, host beetles were dissected on a 2.0% agar plate, after which the plate was kept at room temperature for several weeks. Nematodes proliferated on bacteria associated with the host beetle cadavers. Individuals were thereafter transferred to nematode growth medium (NGM) agar plates seeded with *Escherichia coli* OP50, and have been since kept in laboratory culture on this medium.

**Morphological observation and preparation of type material:** One- to two-week-old cultures of *P. fissidentatus* n. sp. (strain RS5133) and *P. elegans* n. sp. (RS5229) provided material for morphological observation. Observations by light microscopy (LM) were conducted using live nematodes, which were hand-picked from culture plates. Because of the clarity and integrity of its morphology, live material was also used for morphometrics. To generate additional *P. fissidentatus* n. sp. males for observation and type specimens, crosses were set up between 2-5 males and one hermaphrodite on plates with a small bacterial lawn (25 µL OP50 in L-broth). Both F1 males and spontaneous (= fatherless) males were used for study. To prepare type material, nematodes were isolated from type strain cultures, rinsed in distilled water to remove bacteria, heat killed at 65°C, fixed in 5% formalin, and processed through a glycerol and ethanol series using Seinhorst's method (Hooper, 1986).

**Molecular characterization:** To confirm that all isolates of *P. fissidentatus* n. sp. belong to the same species, an 830-bp fragment of the small subunit (SSU) rRNA gene was extracted, amplified, and sequenced for each isolate. Information regarding primers and PCR conditions is given in Mayer et al. (2007). Sequences of the SSU rRNA fragment were identical for all isolates of *P. fissidentatus* n. sp. and are not discussed further herein. The partial SSU rRNA sequences for the type strains of *P. fissidentatus* n. sp. and *P. elegans* n. sp. were published by Mayer et al. (2007) and deposited in the GenBank database under the accession numbers EF623996 and EF634510, respectively.

***Pristionchus fissidentatus*\* n. sp.**

= *Pristionchus* sp. 10 *apud* Mayer et al. (2007, 2009),  
Kanzaki et al. (2012a, b)  
(Figs. 1–4)

**Measurements:**

See Table 1.

**Description:**

**Adults:** Species androdieocious, i.e. consisting of males and self-fertile hermaphrodites. 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 apparent 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 eurytostomatous (wide mouth) forms occurring in both males and females. Dorsal pharyngeal gland clearly observed, penetrating dorsal tooth to gland opening. Anterior part of pharynx (= pro- and metacorpus) 1.5 times as long as posterior part (isthmus and basal bulb). Procorpus very muscular, stout, occupying half to two-thirds of corresponding body diam. Metacorpus 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 or slightly more anterior part of isthmus. Excretory pore not conspicuous, ventrally located with variable position, often slightly anterior to basal bulb to pharyngo-intestinal junction. Hemizonid not clearly observed. Deirid observed laterally, slightly posterior to pharyngo-intestinal junction. Postdeirids present and observed laterally, with positions inconsistent among individuals, 5-8 for males and 9-10 for females being confirmed by LM observation.

**Stenostomatous form:** Cheilostom consisting of six per- and interrational plates. Incision between plates not easily distinguished by LM observation. Anterior end of each plate rounded and elongated to stick out from stomatal opening and form a small flap. Gymnostom short, cuticular ring-like anterior end overlapping with cheilostom internally. Dorsal gymnostomatal wall slightly thickened compared with ventral side. Stegostom bearing: a conspicuous dorsal tooth in the shape of an inverted "V" or, in some cases, not clearly pointed and thus forming an inverted "C"; two bump-like (blunt) left subventral denticles apparently projecting from a common cuticular plate and a separate, prominent denticle slightly left of ventral; a right subventral ridge giving rise to three smooth to conspicuous cusps. Dorsal tooth with strongly sclerotized surface.

**Eurytostomatous form:** Cheilostom divided into six distinct per- and interrational plates. Anterior end of each plate rounded and elongated to stick out from stomatal opening and form a small flap. Gymnostom with thick cuticle, forming a short, ring-like tube. Anterior end of gymnostom overlapping internally with posterior end of cheilostomatal plates. Stegostom bearing: a large claw-like dorsal tooth; a row of left subventral denticles of varying numbers and size, i.e. two large denticles to six small denticles; a right subventral ridge with multiple peaks, and a strong, triangular, tooth-like projection most ventrally. Dorsal tooth movable. Left subventral denticles and right subventral ridge immovable.

\* The species epithet is a compound adjective derived from Latin *fissus* ("split") + participial *dentatus* ("toothed") and refers to the stoma of both mouth forms, in which the right subventral ridge distinctively hosts multiple cusps.



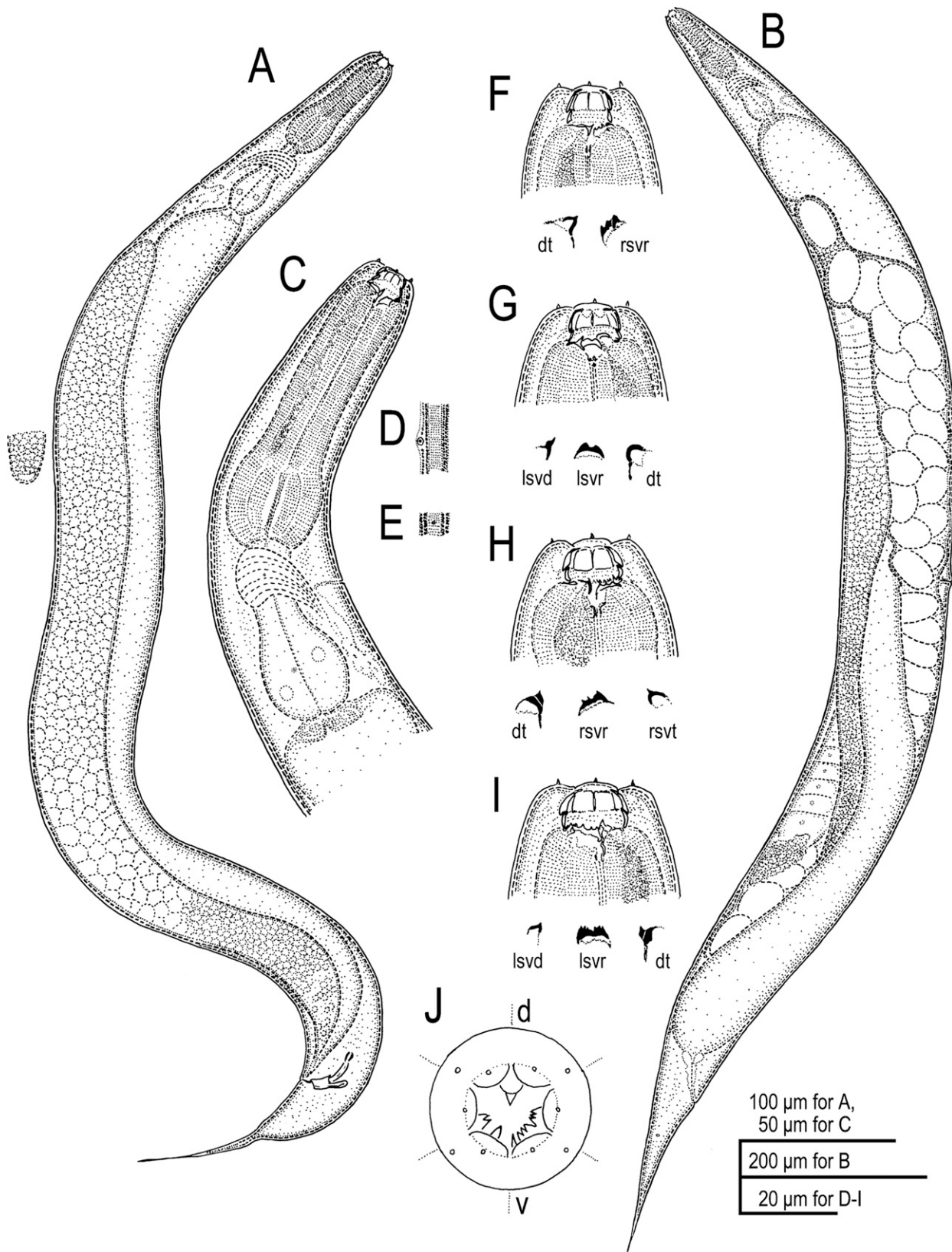


FIG. 1. Adults of *Pristionchus fissidentatus* n. sp. A: Male and the tip of reflexed testis (immediately left of its corresponding position in body) in left lateral view; B: mature hermaphrodite in right lateral view; C: anterior region of adult in right lateral view; D: deirid, lateral field, and body surface pattern, from position near pharyngo-intestinal junction; E: postdeirid; F-I: stomatal morphology and teeth (F: right lateral view of stenostomatous form; G: left lateral view of stenostomatous form; H: right lateral view of eury stomatous form; I: left lateral view of eury stomatous form); J: schematic drawing of *en face* view of eury stomatous male. Abbreviations: dt = dorsal tooth; lsvd = left subventral denticle; lsrv = left subventral ridge; rsvr = right subventral ridge; rsvt = right subventral tooth.

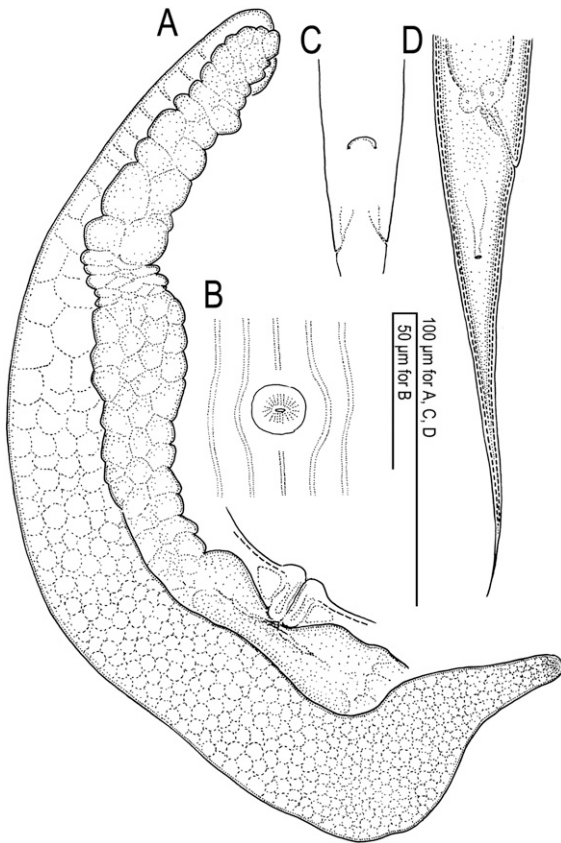


FIG. 2. Adult hermaphrodite of *Pristionchus fissidentatus* n. sp. A: Gonad of young individual in right lateral view; B: vulva in ventral view; C: anus and phasmids in ventral view; D: tail region in right lateral view.

**Male:** Spontaneous males rare (<1%) in culture. 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 two to five rows in reflexed part, then well-developed spermatocytes arranged as two to five 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 manubrium present at anterior end; lamina/calomus complex expanded just posterior to manubrium, then smoothly tapering to pointed distal end. Gubernaculum conspicuous, about one third of spicule in length, relatively broad anteriorly such that dorsal and ventral walls separate at a 45° angle at posterior end. Anterior half of gubernaculum with ear-like shape in lateral view, posterior half forming a tube-like process enveloping spicules. Dorsal side of gubernaculum possessing a single, membranous, anteriorly directed process and a lateral pair of more sclerotized,

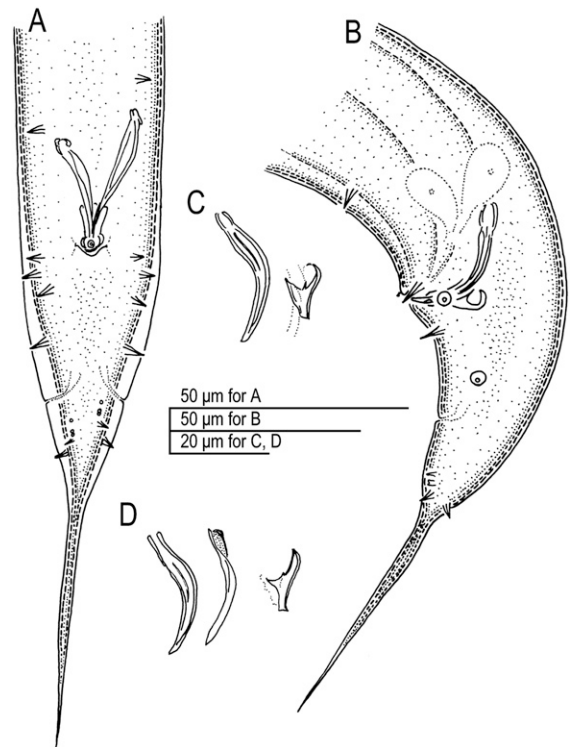


FIG. 3. Adult male of *Pristionchus fissidentatus* n. sp. A: Tail region in ventral view; B: tail region in left lateral view; C, D: left lateral view of spicule and gubernaculum of different individuals.

anteriorly directed processes. Tail conical, with long spike, which has filiform distal end. Thick cuticle 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 the anterior cloacal lip. Nine pairs of genital papillae and a pair of phasmids present and arranged as <P1, (C, P2), P3d, P4, P5d, Ph, (P6, P7, P8), P9d> (= <v1, (C, v2), v3d, v4, ad, Ph, (v5, v6, v7), pd> in nomenclature of Sudhaus and Fürst von Lieven, 2003). Positions of P1 papillae asymmetrical and located about one cloacal body diam. posterior to cloacal slit, C-P4 close to each other (clustered within a half cloacal body diam. length), P5d located middle of P4 and P6 or a half cloacal diam. posterior to P4, Ph around middle between P5d and P6 or close to P6, P6 located one cloacal body diam. or less posterior to P5d, P6-P8 linearly arranged, and P9d almost overlapping with or slightly posterior to P8. P1-P5d papillae of almost equal size, rather large and conspicuous, P6 and P7 very small, sometimes difficult to observe by LM, P8 and P9d small but larger than P6 and P7, i.e. intermediate between P1-P5d and P6/P7 in size. Tip of P7 papillae split into two small papilla-like projections. Tail spike about 1.5–2 cloacal body diam. long. Bursa or bursal flap absent.

**Hermaphrodite:** Relaxed or slightly ventrally arcuate when killed by heat. Gonad didelphic, amphidelphic. Each gonadal system arranged from vulva/vagina as uterus, oviduct, and ovotestis. Anterior gonad right of intestine, with uterus and oviduct extending ventrally

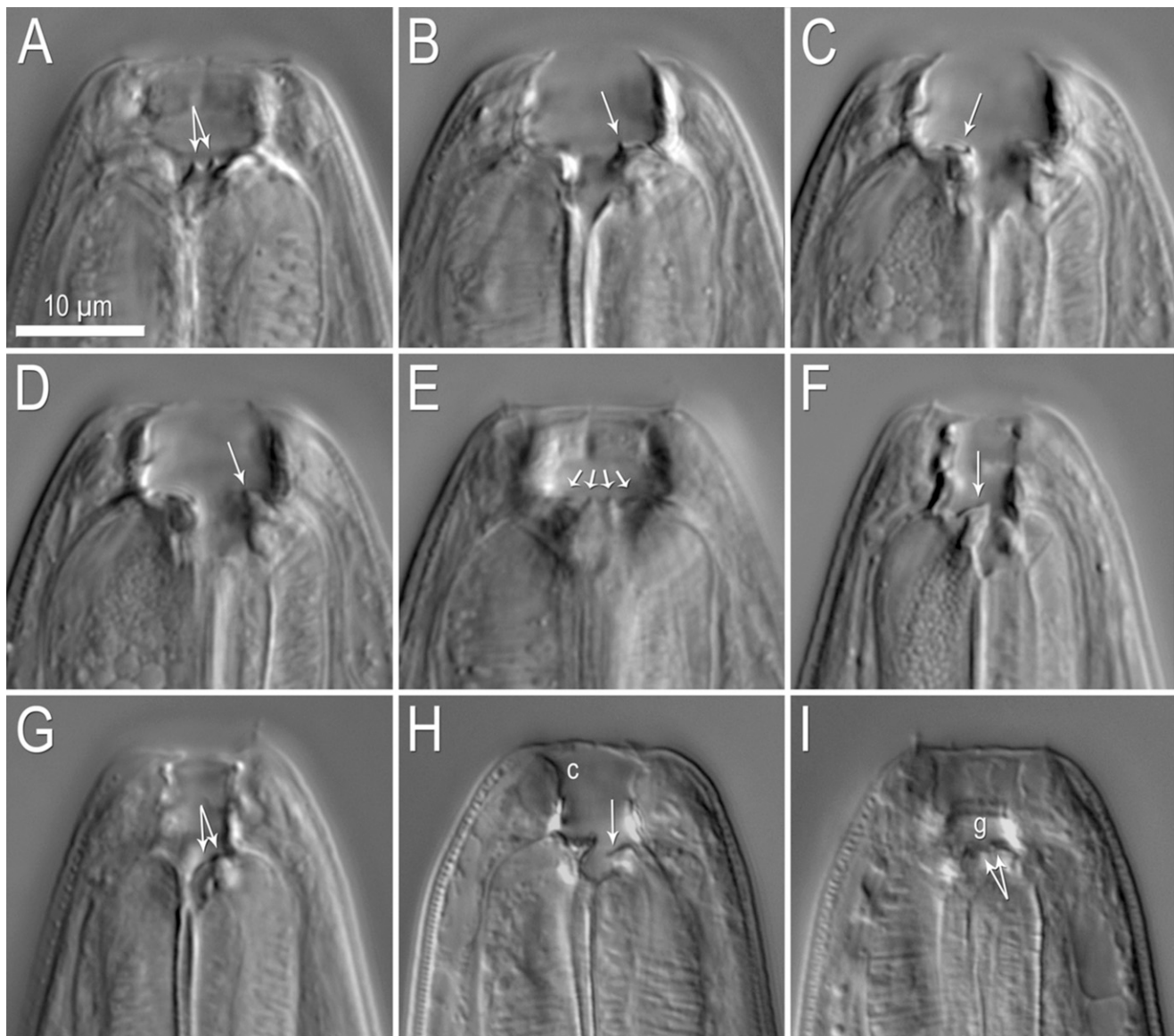


FIG. 4. Nomarski micrographs of the stomatal region of *Pristionchus fissidentatus* n. sp. (A-G) and *P. elegans* n. sp. (H, I). Dorsal is left in all images, which were captured from a right lateral aspect. All images are at same scale. (A-E) show a single eury stomatous female, progressing in longitudinal planes from right of body to left. A: Right subventral ridge, hosting peaks (arrows); B: right subventral tooth (arrow); C: dorsal tooth (arrow); D: left subventral denticle (arrow); E: subventral ridge, hosting multiple peaks (arrows). (F, G) show a single stenostomatous female. F: Dorsal plane, showing dorsal tooth (arrow); G: right subventral plane, showing right subventral ridge, including peaks (arrows). (H, I) show a single stenostomatous. H: Dorsal plane, showing dorsal tooth (arrow), part of right subventral denticle (c); I: left subventral plane, showing left subventral ridge with minute peaks (arrows), the serrated anterior margin of gymnostom (g), and vacuolated cheilostom with dorsal, ventral, and two right sublateral bulges.

and anteriorly on right of intestine and with a totally reflexed (= antidromous reflexion) ovotestis extending dorsally on left of intestine. Oocytes mostly arranged in multiple, sometimes more than 10 rows in distal 2/3 of ovotestis and in single row in remaining 1/3 of ovotestis, one well-developed oocyte present at level just anterior to junction of ovotestis and oviduct, distal tips of each ovotestis reaching the oviduct of opposite gonad branch. Middle part of oviduct serving as spermatheca, self sperm observed in distal part of oviduct, close to ovotestis. Eggs in single to multiple-cell stage or even further developed at proximal part of oviduct (= uterus).

Well-matured hermaphrodite with more than 30 eggs in total. *Receptaculum seminis* not observed. Dorsal wall of uterus at the level of vulva thickened and appears dark in LM observation. Vaginal glands present but obscure. Vagina perpendicular to body surface, surrounded by sclerotized tissue. Vulva slightly protuberant in lateral view, pore-like in ventral view. Rectum about one anal body diam. long, intestinal-rectal junction surrounded by well-developed sphincter muscle. Three anal glands present but not obvious. Anus in form of dome-shaped slit, posterior anal lip slightly protuberant. Phasmid about two anal body diam.



TABLE 1. Morphometrics of eury stomatous hermaphrodite holotype (in glycerin) and hermaphrodite and male specimens of *Pristionchus fissidentatus* n. sp. (temporary water mounts). All measurements made in  $\mu\text{m}$  and in the form: mean  $\pm$  sd (range), except for eury stomatous males, for which all measurements are given.

Character	Eury stomatous hermaphrodite		Stenostomatous hermaphrodite		Stenostomatous male		Eury stomatous male	
	Holotype	Temporary water mounts	Temporary water mounts	Temporary water mounts	Temporary water mounts	Temporary water mounts	Temporary water mounts	Temporary water mounts
n	-	10	12	10	10	3		
L	1439	1300 $\pm$ 121 (1031-1436)	1208 $\pm$ 159 (959-1390)	837 $\pm$ 72 (744-961)	633, 646, 691			
L'	1295	1143 $\pm$ 117 (885-1294)	1055 $\pm$ 148 (813-1234)	716 $\pm$ 73 (623-855)	527, 530, 576			
a	13	16 $\pm$ 0.6 (15-17)	16 $\pm$ 1.4 (15-20)	14 $\pm$ 2.1 (11-18)	10.7, 12.9, 13.5			
b	11	8.5 $\pm$ 0.9 (6.5-9.6)	7.8 $\pm$ 0.9 (6.4-8.8)	6.1 $\pm$ 0.5 (5.0-6.8)	4.2, 4.4, 4.9			
c	10	8.3 $\pm$ 0.9 (7.1-10.1)	8.0 $\pm$ 0.6 (6.4-9.1)	7.0 $\pm$ 0.9 (6.1-9.1)	6.0, 5.6, 6.0			
c'	4.4	4.8 $\pm$ 0.5 (4.2-5.6)	4.9 $\pm$ 0.8 (4.2-7.1)	3.5 $\pm$ 0.4 (3.0-4.0)	2.9, 3.5, 3.3			
T or V	50	50 $\pm$ 1.2 (49-53)	50 $\pm$ 3.0 (44-56)	40 $\pm$ 2.6 (36-44)	54, 52, 53			
Maximum body diam.	107	81 $\pm$ 6.6 (68-90)	75 $\pm$ 14 (53-92)	62 $\pm$ 9.9 (42-76)	59, 50, 51			
Stoma (cheilo- + gymnostom)	6.5	8.5 $\pm$ 1.0 (6.8-9.9)	8.3 $\pm$ 0.8 (7.1-9.4)	7.3 $\pm$ 0.7 (5.4-8.0)	5.3, 5.7, 4.9			
Neck length (head to base of pharynx)	141	153 $\pm$ 4.9 (144-158)	155 $\pm$ 3.9 (150-161)	137 $\pm$ 8.1 (125-150)	151, 148, 140			
Anterior pharynx (pro- + metacarpus)	77	79 $\pm$ 2.5 (75-82)	82 $\pm$ 3.3 (77-86)	74 $\pm$ 6.4 (66-88)	69, 71, 68			
Posterior pharynx (isthmus + basal bulb)	57	65 $\pm$ 3.8 (57-69)	64 $\pm$ 4.8 (57-71)	56 $\pm$ 3.3 (50-59)	58, 54, 51			
Post./ant. pharynx ratio	74	82 $\pm$ 5.2 (73-92)	79 $\pm$ 8.3 (66-92)	75 $\pm$ 5.9 (63-81)	84, 76, 75			
Excretory pore from ant. end	157	163 $\pm$ 12 (140-178)	156 $\pm$ 12 (136-173)	132 $\pm$ 8.9 (119-149)	106, 110, 120			
Testis length	-	-	-	506 $\pm$ 62 (354-571)	343, 333, 367			
Ant. female gonad (with flexure)	992	791 $\pm$ 120 (552-1002)	679 $\pm$ 149 (456-960)	-	-			
Post. female gonad (with flexure)	1259	770 $\pm$ 87 (669-971)	716 $\pm$ 139 (523-900)	-	-			
Vulva to anus distance	581	491 $\pm$ 52 (372-544)	446 $\pm$ 67 (345-543)	-	-			
Cloacal or anal body diam.	29	33 $\pm$ 4.2 (26-40)	32 $\pm$ 3.7 (23-38)	40 $\pm$ 2.6 (36-44)	37, 33, 35			
Tail length	152	158 $\pm$ 14 (142-186)	153 $\pm$ 18 (123-179)	120 $\pm$ 9.4 (106-137)	106, 116, 115			
Spicule length (curve)	-	-	-	40 $\pm$ 2.6 (36-44)	33, 36, 34			
Spicule length (chord)	-	-	-	35 $\pm$ 1.6 (33-37)	29, 33, 31			
Gubernaculum length	-	-	-	15 $\pm$ 1.1 (13-17)	14, 15, 14			

posterior to anus. Tail long, distal end variable from filiform to long and conical.

*Type locality:* The culture from which the type specimens were obtained was originally isolated from soil collected by Iris Dinkelacker in Tatopani, Annapurna area, Myagdi, Nepal in October 2005.

*Distribution and carrier range:* Besides its collection from the type locality, the species was isolated repeatedly by M. Herrmann on La Réunion Island: (1) from soil from Piton Maito in January 2009; (2) from an adult *Adoretus* sp. (Coleoptera: Scarabaeidae) from Basse Vallée in January 2009; (3) from an adult *Oryctes borbonicus* (Coleoptera: Scarabaeidae) from Trois Bassins in January 2010. Although the former two strains were lost, the strain from *O. borbonicus* is available in living culture under culture code RSA024.

*Type material:* Holotype eury stomatous hermaphrodite (slide accession number 30694), four paratype eury stomatous hermaphrodites, four paratype stenostomatous hermaphrodites, three paratype stenostomatous males, and two paratype eury stomatous males (30695–30707) deposited in the University of California Riverside Nematode Collection (UCRNC), Riverside, CA, USA. Three paratype eury stomatous hermaphrodites, three paratype stenostomatous hermaphrodites, and one paratype stenostomatous male

(SMNK-Nema-T 0164 – SMNK-Nema-T 0170) deposited in the Natural History Museum Karlsruhe, Germany. Three paratype eury stomatous hermaphrodites, three paratype stenostomatous hermaphrodites, and one paratype stenostomatous male (SMNH Type-8290 – SMNH Type-8289) deposited in the Swedish Natural History Museum, Stockholm, Sweden.

*Type strain culture:* Available in living culture and as frozen stocks under culture code RS5133 in the Department of Evolutionary Biology, Max Planck Institute for Developmental Biology, Tübingen, Germany and can be provided to other researchers upon request.

*Diagnosis and relationships:* Besides its generic characters, *Pristionchus fissidentatus* n. sp. is diagnosed by its unique stomatal armature: the stenostomatous form has a right subventral ridge with three cusps vs. no or one small denticle; left subventral sector with a prominent denticle slightly left of ventral in addition to two blunt cusps arising from a common cuticular plate vs. left subventral sector with no prominent denticle. Distinguishing *P. fissidentatus* n. sp. in the eury stomatous form is a right subventral ridge with multiple peaks and a narrow tooth-like projection ventrally vs. a single claw-like right subventral tooth; a left subventral plate with a pointed, tooth-like projection ventrally vs. no tooth-like projection ventrally. The species is diagnosed by the

size and arrangement of male genital papillae arranged as <P1, (C, P2), P3d, P4, P5d, Ph, (P6, P7, P8), P9d>, whereby only one pair (P1) is located anterior to cloacal opening, and cloaca and P2-P4 are located very close to each other. Distinguishing *P. fissidentatus* n. sp. from its congeners is a gubernaculum that is relatively short (one third of spicule length) and broad anteriorly vs. longer (half of spicule length) and tapering smoothly to anterior end. The species is distinguished from the following species by a hermaphroditic vs. gonochoristic mode of reproduction: its putative, distant phylogenetic neighbor *P. elegans* n. sp. (Mayer et al., 2007, 2009); most other described *Pristionchus* species, including the morphologically and molecularly characterized species *P. aerivorus* (Cobb in Merrill and Ford, 1916) Chitwood, 1937 and *P. uniformis* Fedorko and Stanuszek, 1971; the molecularly and biologically circumscribed species *P. americanus*, *P. marianneae*, *P. pauli*, and *P. pseud aerivorus*.

***Pristionchus elegans*\* n. sp.**

= *Pristionchus* sp. 15 *apud* Mayer et al. (2007, 2009),  
Kanzaki et al. (2012a, b)  
(Figs. 4–6)

**Measurements:**

See Table 2.

**Description:**

**Adults:** 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 apparent 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. Stenostomatous (narrow mouth) occurring in both males and females. Eurystomatous (wide mouth) form not observed. Dorsal pharyngeal gland clearly observed, penetrating dorsal tooth to gland opening. Anterior part of pharynx (= pro- and metacarpus) 1.5 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 region of isthmus. Excretory pore not conspicuous, ventrally located with variable position, often at level of basal bulb to pharyngo-intestinal junction. Hemizonid not clearly observed. Deirid observed laterally, slightly posterior to pharyngo-intestinal junction. Postdeirids present and observed laterally, with positions inconsistent among

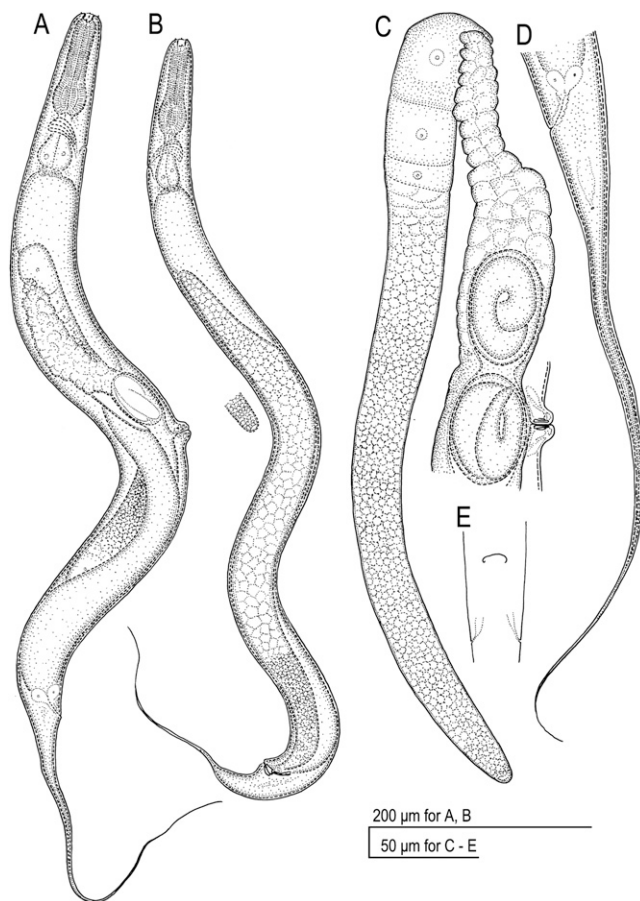


FIG. 5. Adults of *Pristionchus elegans* n. sp. A: Female in right lateral view; B: male and reflexed testis tip in left lateral view; C: anterior female gonad in right lateral view; D: female tail region in left lateral view; E: female anus and phasmids in ventral view.

individuals, 5-8 for males and 9-10 for females being confirmed by LM observation.

**Stenostomatous form:** Cheilostom consisting of six per- and interradiial plates. Incision between plates not easily distinguished by LM observation. Cheilostomatal epidermis appearing vacuolated and to push cheilostomatal walls inward (medially) such that cheilostomatal part of stoma is often narrowest at middle region of cheilostom. Vacuolated region bulges in sectors corresponding to six cheilostomatal plates. Anterior end of each plate rounded and elongated to stick out from stomatal opening and form a small flap. Gymnostom short, shorter than or almost same depth as cheilostom, cuticular ring-like anterior end overlapping with cheilostom internally. Anterior margin of gymnostomatal tube (capsule) conspicuously serrated. Stegostom bearing a conspicuous dorsal tooth in the shape of an inverted “V”, usually with a row of three closely positioned and minute left subventral denticles, or sometimes a blunt ridge (no denticles), apparently projecting from a common, narrow cuticular plate, and a right subventral ridge with a single bluntly pointed or bump-like denticle. Dorsal tooth with strongly sclerotized surface.

\* The species epithet is a Latin adjective, corresponding to the English, and refers to the swift, graceful locomotion of this nematode in live samples and culture.



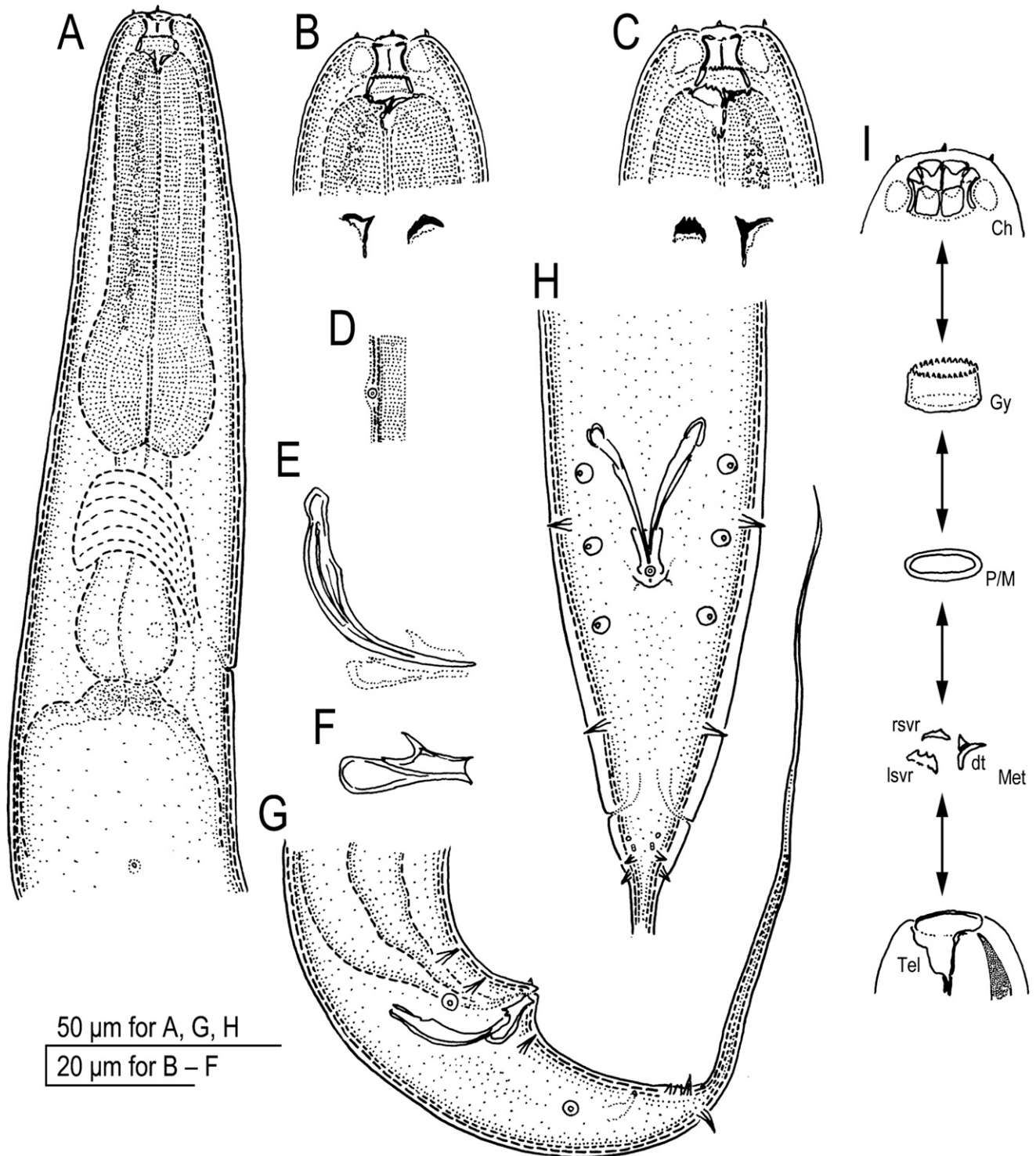


FIG. 6. Adults of *Pristionchus elegans* n. sp. A: Anterior part of adult in right lateral view; B, C: stoma and teeth morphology of stenostomatous form (B: right lateral view; C: left lateral view); D: deirid, lateral field and body surface pattern; E, F: spicule and gubernaculum in right lateral view; G, H: male tail region (G: right lateral view; H: ventral view); I: schematic drawing of left lateral view of stoma. Abbreviations: Ch = cheilostom; Gy = gymnostom; P/M = pro-meso stegostom (assumed to be degenerated ring surrounding metastegostomatal elements, not clearly visible); Met = metastegostomatal teeth and denticles; rsvr = right subventral ridge or denticle; lsvr = left subventral ridge; dt = dorsal tooth; Tel = telostegostom.

*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 two or four rows in reflexed part, then

well-developed spermatocytes arranged as three to five 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

TABLE 2. Morphometrics of stenostomatous male holotype (in glycerin) and male and female specimens of *Pristionchus elegans* n. sp. (temporary water mounts). All measurements made in  $\mu\text{m}$  and in the form: mean  $\pm$  sd (range).

Character	Stenostomatous male		Stenostomatous female
	Holotype	Temporary water mounts	Temporary water mounts
n	-	20	20
L	766	950 $\pm$ 85 (730-1067)	1379 $\pm$ 115 (1155-1602)
L'	603	765 $\pm$ 77 (610-882)	1048 $\pm$ 103 (848-1281)
a	23	19 $\pm$ 2.1 (16-23)	14 $\pm$ 1.3 (11-16)
b	7	8.1 $\pm$ 0.6 (7.0-9.0)	9.9 $\pm$ 0.7 (8.5-11)
c	4.7	5.2 $\pm$ 0.6 (4.4-6.7)	4.2 $\pm$ 0.7 (3.6-7.0)
c'	7.1	5.8 $\pm$ 0.8 (5.4-7.0)	9.9 $\pm$ 1.8 (5.5-13)
T or V	58	63 $\pm$ 3.7 (57-72)	42 $\pm$ 1.7 (40-48)
Maximum body diam.	34	51 $\pm$ 7.3 (41-65)	98 $\pm$ 13 (84-132)
Stoma (cheilo- + gymnoston)	6.1	7.6 $\pm$ 0.6 (6.6-9.1)	9.4 $\pm$ 0.7 (7.8-11)
Neck length (head to base of pharynx)	116	118 $\pm$ 7.4 (104-129)	139 $\pm$ 9.0 (126-165)
Anterior pharynx (pro- + metacarpus)	69	67 $\pm$ 4.8 (58-74)	79 $\pm$ 5.3 (73-95)
Posterior pharynx (isthmus + basal bulb)	41	44 $\pm$ 4.1 (38-54)	50 $\pm$ 4.3 (45-61)
Post./ant. pharynx ratio	59	67 $\pm$ 5.3 (59-82)	64 $\pm$ 4.6 (55-77)
Excretory pore from ant. end	110	137 $\pm$ 9.1 (122-150)	159 $\pm$ 10 (136-177)
Testis length	446	603 $\pm$ 73 (470-749)	-
Ant. female gonad (with flexure)	-	-	705 $\pm$ 77 (608-921)
Post. female gonad (with flexure)	-	-	706 $\pm$ 65 (563-846)
Vulva to anus distace	-	-	467 $\pm$ 53 (355-580)
Cloacal or anal body diam.	23	32 $\pm$ 2.1 (27-36)	43 $\pm$ 5.0 (27-42)
Tail length	163	186 $\pm$ 21 (120-216)	331 $\pm$ 43 (175-381)
Spicule length (curve)	36	36 $\pm$ 2.6 (29-39)	-
Spicule length (chord)	30	30 $\pm$ 3.3 (21-35)	-
Gubernaculum length	14	14 $\pm$ 0.9 (13-16)	-

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, narrow and squared manubrium present at anterior end; lamina/calomus complex clearly expanded 1/3 length from anterior end, then smoothly tapering to pointed distal end. Gubernaculum conspicuous, about 2/5 of spicule in length, anterior half with ear-like shape in lateral view, posterior half forming a tube-like process enveloping spicules. Dorsal side of gubernaculum possessing a single, membranous, anteriorly directed process and a lateral pair of more sclerotized, anteriorly directed processes. Tail conical, with long spike, which has filiform distal end. Thick cuticle around tail region, sometimes falsely appearing like a narrow leptoderan bursa in ventral view. Cloacal opening slit-like in ventral view. One small, ventral, single genital papilla on the 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> (= <v1, (v2d, v3), C, v4, ad, Ph, (v5, v6, v7), pd> in nomenclature of Sudhaus and Fürst von Lieven, 2003), where, in many individuals, phasmid (Ph) and P6 are clearly apart. Ph located in the middle of P5d and P6 or closer to P6. P6-P8 arranged in a triangle, i.e. P7 is located slightly ventral compared with P6 and P8, and position of P9d overlapping with or a little posterior to P8. P1-P4 papillae of almost equal size, rather large and con-

spicuous, P5d slightly smaller than P1-P4, P6 and P7 very small, sometimes difficult to observe by LM, P8 and P9d small but larger than P6 and P7, i.e. intermediate between P5d and P6/P7 in size. Tip of P7 papillae split into two small papilla-like projections. Tail spike about four to five cloacal body diam. long. Bursa or bursal flap absent.

*Female*: Relaxed or slightly ventrally arcuate when killed by heat. Gonad didelphic, amphidelphic. Each gonadal system arranged from vulva/vagina as uterus, oviduct, and ovary. Anterior gonad 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 on left of intestine. Oocytes mostly arranged in two to eight rows in distal 2/3 of ovary and in double or single row in rest of ovary, one well-developed oocyte or egg at level just anterior to junction of ovary and oviduct, distal tips of each ovary reaching the oviduct of opposite gonad branch. Middle part of oviduct serving as spermatheca. Eggs in single to multiple-cell stage, or often with young juvenile at proximal part of oviduct (= uterus). *Receptaculum seminis* not observed. Vaginal glands present but obscure. Vagina perpendicular to body surface, surrounded by sclerotized tissue. Vulva slightly protuberant in lateral view, pore-like in ventral view. Rectum about one anal body diam. long, intestinal-rectal junction surrounded by well-developed sphincter muscle. Three anal glands present but not obvious. Anus in form of dome-shaped slit, posterior anal lip slightly protuberant. Phasmid about one to two anal

body diam. posterior to anus. Tail long, distal end variable from filiform to long and conical.

*Type host (carrier) and locality:* The culture from which the type specimens were obtained was originally isolated from the body of an adult *Phelotrupes auratus* (Coleoptera: Scarabaeidae) collected by M. Herrmann in a forest near Kutsuki, Takashima-shi, Shiga Prefecture, Japan (35°2'12.3"N, 135°54'15.5" W) in June 2006.

*Type material:* Holotype stenostomatous male (slide accession number 30708), six paratype stenostomatous males, six paratype stenostomatous hermaphrodites (30709–30720) deposited in the UCRNC, CA, USA. Four paratypes each of stenostomatous males and stenostomatous females (SMNK-Nema-T 0171 – SMNK-Nema-T 0178) deposited in the Natural History Museum Karlsruhe, Germany. Four paratypes each of stenostomatous males and stenostomatous females (SMNH Type 8297 – SMNH Type-8304) deposited in the Swedish Natural History Museum, Stockholm, Sweden.

*Type strain culture:* Available in living culture under culture code RS5229 in the Department of Evolutionary Biology, Max Planck Institute for Developmental Biology, Tübingen, Germany and can be provided to other researchers upon request. Attempts to achieve frozen stocks of this species have been unsuccessful.

*Diagnosis and relationships:* Besides its generic characters, *Pristionchus elegans* n. sp. is diagnosed by the unique stoma shape and armature of its stenostomatous (i.e. the only known) form: the tissue underlying the cheilostom appears vacuolated vs. being indistinct from other surrounding tissue; cheilostomatal walls bend inward (medially), such that the cheilostom is narrowest at its middle, vs. tapering anteriorly with apex of curve being distal; conspicuous serration of anterior margin of gymnostomatal tube; the left subventral serrated plate of the stegostom is narrow and with a row of usually three minute denticles vs. being wider and with two to three large, blunt bumps as in other *Pristionchus* spp. The species is also diagnosed by the size of the male genital papillae and their arrangement as <P1, (P2d, P3), C, P4, P5d, Ph, (P6, P7, P8), P9d>, where P1 is closer to P2 (further posterior) than in other *Pristionchus* species. It is distinguished from its putative, distant phylogenetic neighbor *P. fissidentatus* n. sp. (Mayer et al., 2007, 2009) and from *P. biformis* (Hirschmann, 1951) Sudhaus and Füst von Lieven, 2003, *P. entomophagus* (Steiner, 1928) Sudhaus and Füst von Lieven, 2003, *P. lheritieri* (Maupas, 1919) Paramonov, 1952, *P. maupasi* (Potts, 1910) Paramonov, 1952, and *P. pacificus* by a gonochoristic vs. hermaphroditic mode of reproduction.

#### DISCUSSION

Taxonomy of *Pristionchus* has recently enjoyed a windfall of newly discovered species, although clear distinctions among them are increasingly difficult to

make. Several groups of morphologically indistinguishable species are known, including a clade of the North American species *P. aerivorius*, *P. americanus*, *P. marianneae*, *P. pauli*, and *P. pseudaeivorius* (Herrmann et al., 2006b). The recently described *P. pacificus* species complex contains four known species which are morphologically cryptic, phylogenetically close, and distinguishable primarily by their reproductive isolation from each other (Kanzaki et al., 2012a). Especially problematic is that original descriptions of most valid species of *Pristionchus* do not contain detailed information about their mouthparts, which are generally diverse and diagnostic in diplogastrid taxa (Sudhaus and Füst von Lieven, 2003). Information is limited mostly to the presence and sometimes number of teeth, although even the latter is unclear in older descriptions. For example, the description of *P. entomophagus* mentions three teeth, presumably in reference to two opposing teeth and a left serrated plate, albeit it is unlikely that all three can be seen in any one specimen (Steiner, 1928). Likewise, *P. inermis* (Bütschli, 1874) Paramonov, 1952 was described as having two or possibly three teeth (Bütschli, 1874). Apart from missing information, even detailed observations of *P. lheritieri*, *P. maupasi*, and *P. pacificus* by Füst von Lieven and Sudhaus (2000) give an impression of morphological homogeneity of characters within *Pristionchus*, even when they are dissected to the level of individual peaks on ridges and plates. Synonymization of most *Pristionchus* species based apparently on reproductive mode (Andrássy, 1984) also reflects a lack of distinctive characters. In contrast to the general uniformity reported for stomatal characters in the genus, *P. fissidentatus* n. sp. and *P. elegans* n. sp. are strikingly unique.

The presence of a stenostomatous dorsal tooth shaped like an inverted “V”, distinct from a claw-like dorsal tooth in the eurytostomatous form where that form is known, is consistent across the genus and is observed in the new species. However, other stomatal characters show greater differences. A strong deviation in *P. fissidentatus* n. sp. from the general pattern of *Pristionchus* is in the subventral sectors of the stenostomatous stegostom. In addition to having two to three blunt peaks as in all other nominal *Pristionchus* species characterized in enough detail for adequate comparison (i.e. *P. arcanus*, *P. exspectatus*, *P. japonicus*, *P. lheritieri*, *P. maupasi*, *P. pacificus*, and *P. elegans* n. sp.) (Füst von Lieven and Sudhaus, 2000; Kanzaki et al., 2012a), the left subventral sector has a separate, additional strongly projecting denticle. The right subventral ridge, which is generally smooth or ornamented with a small denticle in *Pristionchus* (Füst von Lieven and Sudhaus, 2000; Sudhaus and Füst von Lieven, 2003), is armed with three cusps, reminiscent of the left subventral plate in either mouth form of other *Pristionchus* species (Sudhaus and Füst von Lieven, 2003). The eurytostomatous form also shows a right subventral ridge with multiple peaks instead of a tooth with



a single cusp as typical for diplogastrids with subventral teeth. Thus both subventral stegostomatal sectors in both mouth-forms are topographically complex, in contrast to the left-biased complexity of other diplogastrid species with complex stomata. Still another layer of complexity is seen in the additional tooth-like projections in both subventral sectors of the eury stomatous stegostom.

In *P. elegans* n. sp. the most distinctive region is the cheilostom, the wall of which bulges inward and is underlain by a transparent ring of material resembling a vacuole in the transverse plane. This contrasts with the cheilostom of either mouth form in all other *Pristionchus* species or in those of other diplogastrid genera, which lacks any such ring (Fürst von Lieven and Sudhaus, 2000). Detailed examination of the unusual, diagnostic cheilostomatal wall, given the presumed conservation of epidermal architecture in this part of the stoma (Ragsdale and Baldwin, 2010), could give insight into formation processes or character evolution of the diplogastrid cheilostom. The stegostom of *P. elegans* n. sp. also differs from the stenostomatous form of other *Pristionchus* species by the general reduction of its features, such as in the narrowing of the left subventral plate and its associated serration. The potential for variability in the stegostom, which is observed in both new species, should be given close attention in future descriptions in the genus.

Besides stoma characters, male genital papillae are also increasingly informative for diagnosing species or clades of *Pristionchus*. In a review of the descriptive literature, Kanzaki et al. (2012a) found the relative positions of P1-P4 and cloacal opening to be divergent and informative among species. These four papillae showed greatest differences among species, especially with regard to intraspecific variation, although the arrangement of P6-P8 was also informative among members of the *P. pacificus* species complex. Whether P2 and P3 were clearly apart along the main body axis divided species into two groups, although these groups did not seem to correlate with independently derived phylogeny (e.g. Mayer et al., 2007, 2009; Kanzaki et al., 2012a). Further variation of anterior genital papillae is reported herein for *P. elegans* n. sp., in which P1 is relatively far posterior, closing the distance between P1 and P2 in comparison to other species of *Pristionchus*. Papilla P1 in *P. fissidentatus* n. sp. shows an anterior position more typical of *Pristionchus*, although the species uniquely shows close proximity of P2-P4. The divergence in genital papillae arrangement demonstrates their potential usefulness as characters for diagnosis of *Pristionchus*.

The basal phylogenetic positions of *P. fissidentatus* n. sp. and *P. elegans* n. sp. (Mayer et al., 2007, 2009) make the species useful for reconstructing ancestral character states of more apical taxa, such as the lineage including the *P. pacificus* species complex. In addition to morphological characters, which are given additional complexity by the new species, other biological phenomena can also be put in a more thorough compar-

ative context. With regard to biogeography, the range of *P. fissidentatus* n. sp. and *P. elegans* n. sp. including Nepal and Japan, respectively, suggests an ancestral Asian distribution for the genus (Mayer et al., 2007). This is supported by Japanese ranges of other taxa close to the root of the genus: the closely related, more apical *Pristionchus* sp. 14 (Mayer et al., 2007, 2009) and the closest known outgroup to the genus, *Parapristionchus giblindavisi* (Kanzaki et al., 2011, 2012b). In addition to a South and East Asian distribution of basal taxa in *Pristionchus*, *P. fissidentatus* n. sp. has also been collected on La Réunion Island in the Indian Ocean. Although geographic data for nematodes are chronically incomplete, the wide or disjunct distribution known for *P. fissidentatus* n. sp. is in line with the intuitive hypothesis that hermaphroditic species can more easily disperse and colonize new ranges than can gonochorists (Herrmann et al., 2010). This hypothesis is supported by the widespread distributions of the hermaphroditic species *P. entomophagus* (Steiner, 1928; Herrmann et al., 2006a, 2006b) and *P. pacificus* (Herrmann et al., 2010), the latter being much more easily recovered in sampling than closely related gonochorists (Kanzaki et al., 2012a). The causal link between reproductive mode and dispersal is necessarily elusive, but the upheld correlation between the two is noteworthy.

A final remark on the relevance of new *Pristionchus* species is that they hold implications for the evolution of phenotypic plasticity. Namely, we report the putative absence (or rarity) of a eury stomatous form in *P. elegans* n. sp. The extreme bias of the stomatal dimorphism in this species reveals variability of the plasticity, which is of special importance given the adoption of *P. pacificus* as a model for studying phenotypic plasticity in general (Bento et al., 2010). The dimorphism is known to differ among genera of Diplogastridae (Kiontke and Fitch, 2010). However, the extent to which the dimorphism varies among closely related species is still unknown. The absence of plasticity has been reported for the aptly named *P. uniformis* (Fedorko and Stanuszek, 1971), standing in contrast to the genus diagnosis, which includes presence of a stomatal dimorphism (Sudhaus and Fürst von Lieven, 2003). The extent of variability and how rare the eury stomatous forms really are in apparently monomorphic species remains to be tested.

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