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Source: Zoological Science, 30(8) : 680-692

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zsj.30.680>

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# Two New Species of *Pristionchus* (Nematoda: Diplogastridae) Support the Biogeographic Importance of Japan for the Evolution of the Genus *Pristionchus* and the Model System *P. pacificus*

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*Pristionchus pacificus* Sommer, Carta, Kim, and Sternberg, 1996 is an important model organism in evolutionary biology that integrates developmental biology with ecology and population genetics. This species is part of a sub-complex of the genus *Pristionchus* that is considered to have originated in East Asia. Here, we describe two new species of *Pristionchus*, *P. maxplancki* and *P. quartusdecimus*, which were isolated from beetles in Japan, supporting the hypothesis that a region including Japan is the origin of diversification of the *P. pacificus* species complex. Phylogeny inferred from a partial small subunit rRNA gene and 25 ribosomal protein genes shows *P. maxplancki* to be the closest known outgroup to a triad of sibling species, including *P. pacificus*. *Pristionchus quartusdecimus* is a putative outgroup to the *P. pacificus* species complex, supporting a more ancient origin of *Pristionchus* species in the region. Species diagnoses are based on morphological and molecular characters, in addition to reproductive isolation for *P. maxplancki*. Members of the *P. pacificus* species complex as well as *P. quartusdecimus* are distinguished by stegostomatal structures, male genital papilla arrangement, and gubernaculum shape. The discovery of a new member of the *P. pacificus* species complex allows greater precision in polarizing and reconstructing ancestral states in the comparative model system centering on *P. pacificus*. Together with previous reports, these findings support an important biogeographic role of Japan in the evolution of the genus *Pristionchus* and the *P. pacificus* species complex, especially the associated phenotypic evolution of mouth morphology.

**Key words:** biogeography, Coleoptera, comparative model system, male tail, phylogeny, species description, stoma, taxonomy

## INTRODUCTION

*Pristionchus pacificus* Sommer et al., 1996 has emerged as a model system that allows integration of laboratory-based mechanistic studies in developmental biology and evo-devo with fieldwork, in particular ecology and population genetics. Well-developed forward and reverse genetic tools are available for *P. pacificus*, as are a fully sequenced genome and methods for DNA-mediated transformation (Dieterich et al., 2008; Sommer, 2009). This species is known to occur in a necromenic association with scarab beetles (Herrmann et al., 2007), and extensive sampling has shown La Réunion Island in the Indian Ocean to be a well-

suited microcosm for integrative studies, as it hosts much or most of the phylogenetic diversity within the species (Morgan et al., 2012).

Recent discovery of a cryptic species complex that includes *P. pacificus* (Kanzaki et al., 2012a) has provided an empirical macroevolutionary context to model systems research. Namely, the analytical tools established for *P. pacificus* can be readily applied to sibling species, enabling the investigation of processes of divergence and speciation. For example, a sequenced genome for the putative sister species *P. exspectatus* Kanzaki et al., 2012a, given the reference genome sequence available for *P. pacificus*, has yielded insights into dynamic processes of genome evolution that may have easily been missed when comparing more distantly related species (Rödelsperger et al., unpubl.). Furthermore, the ability of *P. pacificus*, *P. exspectatus*, and *P. arcanus* Kanzaki et al., 2012a to produce viable F1 hybrids facilitates the study of the genetic basis of divergent traits, such as reproductive mode (Kanzaki et al., 2012a).

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doi:10.2108/zsj.30.680

Denser taxon sampling within the *P. pacificus* species complex will thus enable the precise reconstruction of character evolution that is necessary for understanding processes at the interface of micro- and macroevolution.

The genus *Pristionchus* Kreis, 1932, belonging to Diplogastridae Micoletzky, 1922, is known largely from the Holarctic region, which includes the known ranges of all molecularly characterized gonochoristic species in the genus (Herrmann et al., 2006a, b, 2007; Mayer et al., 2007, 2009; Kanzaki et al., 2012a, c). Within this distribution, the most basal known species, *P. elegans* Kanzaki et al., 2012b, and the closest known outgroup to *Pristionchus*, *Parapristionchus giblindavisi* Kanzaki et al., 2012b, were both described from Japan, as were the model *P. pacificus*, its sibling species, and close outgroups (Herrmann et al., 2007; Kanzaki et al., 2012a, c). Current host range information therefore suggests Japan or East Asia to be a center of radiation for *Pristionchus*. Herein, we circumscribe two new species from the region and place them in a phylogenetic context inferred from sequences of the partial small subunit (SSU) rRNA gene and 25 ribosomal protein genes, including original sequence data for one of the new species. Consequences of the new species for morphological diagnosis in the *P. pacificus* species complex are also presented. Together with previous reports, this study supports an important biogeographic role of Japan for the evolution of the genus *Pristionchus* and the *P. pacificus* species complex.

## MATERIALS AND METHODS

### Nematode isolation and cultivation

The new species were isolated from beetles as described in descriptions below and in Herrmann et al. (2007). For both new species, the carrier beetle was 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 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 the two new species 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 used for morphometrics. 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 TAF to a final concentration of 5% formalin and 1.5% triethanolamine, and processed through a glycerol and ethanol series using Seinhorst's method (see Hooper, 1986). Nomarski micrographs were taken using a Zeiss Axio Imager Z.1 microscope and a Spot RT-SE camera supported by the program MetaMorph v.7.1.3 (Molecular Devices, Sunnyvale, CA, USA). In addition to the newly described species, live specimens of *P. arcanus*, *P. exspectatus*, *P. japonicus*, and *P. pacificus*, cultures of which were maintained as described for the two new species, were observed by LM for comparison.

### Molecular characterization and phylogenetic analysis

To diagnose individual species, we amplified and sequenced an approximately 1-kb fragment of the SSU rRNA gene using the primers SSU18A (5'-AAAGATTAAGCCATGCATG-3') and SSU26R (5'-CATTCTTGGCAAATGCTTTTCG-3') (Floyd et al., 2002). The final alignment of the SSU rRNA gene fragment consisted of 851

positions. Phylogenetic analyses were performed on 25 ribosomal protein genes that have been used in previous studies of the genus (Mayer et al., 2007; Kanzaki et al., 2012a). The dataset of ribosomal protein genes comprised a total of 9,855 aligned coding nucleotides. Genes included in analysis were: *rpl-1*, *rpl-2*, *rpl-10*, *rpl-14*, *rpl-16*, *rpl-23*, *rpl-26*, *rpl-27*, *rpl-27a*, *rpl-28*, *rpl-30*, *rpl-31*, *rpl-32*, *rpl-34*, *rpl-35*, *rpl-38*, *rpl-39*, *rps-1*, *rps-8*, *rps-20*, *rps-21*, *rps-24*, *rps-25*, *rps-27*, and *rps-28*. Information on genes, primers, and PCR conditions is given in Mayer et al. (2007). Phylogenetic analysis included all nominal *Pristionchus* species for which both SSU rRNA and ribosomal protein gene sequences were available, the two new species, *Koerneria* sp. RS1982, and two undescribed species (*Pristionchus* sp. 16, *Pristionchus* sp. 25) for which sequences will be presented elsewhere (Kanzaki et al., in press).

The concatenated dataset of the partial SSU rRNA and ribosomal protein genes was aligned using MUSCLE (Edgar, 2004), followed by manual alignment in MEGA5.05 (Tamura et al., 2011) to remove ambiguously aligned positions. The alignment was partitioned four ways: three subsets corresponded to codon positions for the concatenated ribosomal protein genes and one subset contained the partial SSU rRNA gene. Analyses by maximum likelihood (ML) and Bayesian inference, as implemented in RAxML v.7.2.8 (Stamatakis, 2006) and MrBayes 3.2 (Ronquist et al., 2012), respectively, were used to infer the phylogeny. Fifty independent runs were performed for the ML analysis, in which trees were inferred under a general time-reversible model with gamma-shaped distribution of rates across sites. Bootstrap support was calculated by 1000 pseudoreplicates on the most likely tree among all runs. Bayesian analyses were initiated with random starting trees and were run with four chains for  $4 \times 10^6$  generations. Markov chains were sampled at intervals of 100 generations. Two independent runs were performed for the analysis. After confirming convergence of runs and discarding the first  $2 \times 10^6$  generations as burn-in, remaining topologies were used to generate a 50% majority-rule consensus tree with clade credibility values given as posterior probabilities (PP). Bayesian analysis invoked a mixed model of substitution with a gamma-shaped distribution across sites and specified *Koerneria* sp. RS1982 as outgroup. Model parameters were unlinked across character partitions in both ML and Bayesian analyses.

### Mating experiments

Because preliminary diagnosis by SSU rRNA sequence revealed *P. maxplancki* to be close to *P. japonicus*, mating experiments were performed between the two species to test whether they were each biologically unique. In the experiments, five virgin (J4) females of one strain were placed together with five males of the other strain on a plate maintained at 20°C and containing a lawn grown from 25 µl of *Escherichia coli* OP50 in L-Broth. All crosses were performed reciprocally and in triplicate. We considered strains to be reproductively isolated if no viable F1 progeny resulted from the crosses.

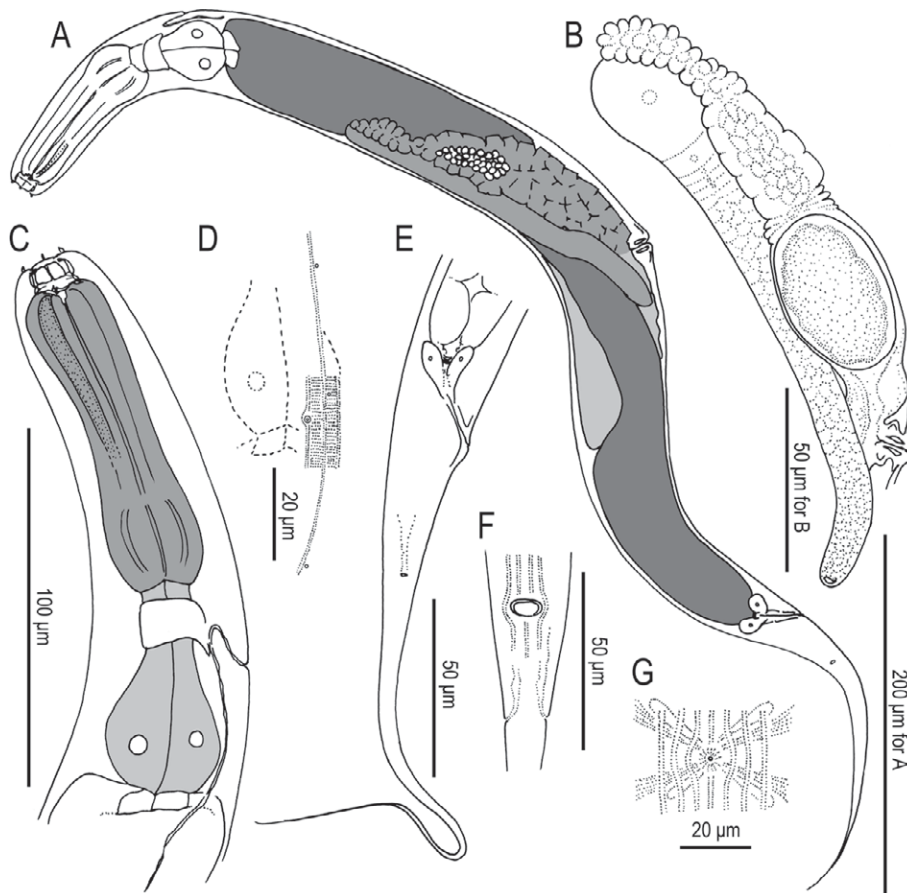
## RESULTS

### Taxonomy

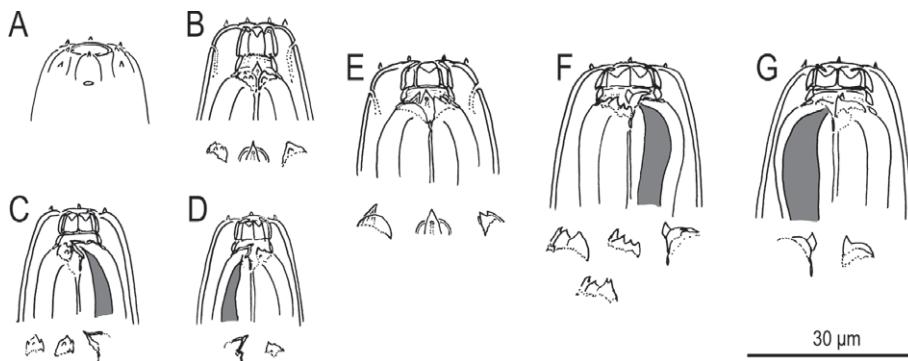
Previous descriptions have shown *Pristionchus* species are generally alike in many morphological characters (Herrmann et al., 2006b; Kanzaki et al., 2012a, c), which is consistent with the findings of the present study (Figs. 1–5). Therefore, to avoid redundancy, characters common to both new species are described first, followed by specific characters and diagnoses for each species.

### Description of common morphological characters

**Adults.** Body cylindrical, stout (Figs. 1A, 3A, 4A, B). Cuticle thick, with fine annulation and clear longitudinal stri-



**Fig. 1.** Adult female of *Pristionchus maxplancki* n. sp. Drawings are of live specimens (non-types) from temporary mounts. **(A)** Whole body of eury stomatous individual, right lateral view. **(B)** Anterior reproductive tract, right lateral view. **(C)** Neck region of eury stomatous female, right lateral view. **(D)** Deirid, with "postdeirid" and "predeirid" pores, left lateral view. **(E)** Tail region, left lateral view. **(F)** Anus, ventral view. **(G)** Vulva, ventral view.



**Fig. 2.** Stomatal region of *Pristionchus maxplancki* n. sp. Drawings are of live specimens (non-types) from temporary mounts. **(A)** Lip region of stenostomatous male, lateral view. **(B)** Stenostomatous female, ventral view. Below are right subventral denticles, dorsal tooth, and left subventral denticles. **(C)** Stenostomatous female, left lateral view. Below are two variants of left subventral denticles (left, center) and dorsal tooth (right). **(D)** Stenostomatous female, right lateral view. Below are dorsal tooth and right subventral denticles. **(E)** Eury stomatous female, ventral view; below are right subventral tooth, dorsal tooth, and left subventral denticles. **(F)** Eury stomatous female, left lateral view; below are three variants of left subventral denticles (left) and dorsal tooth (right). **(G)** Eury stomatous female, right lateral view; below are dorsal tooth and right subventral tooth.

ations. Lateral field consisting of two lines, only weakly distinguishable from body striation. Head without apparent lips, and with six short and papilliform labial sensillae. Four small,

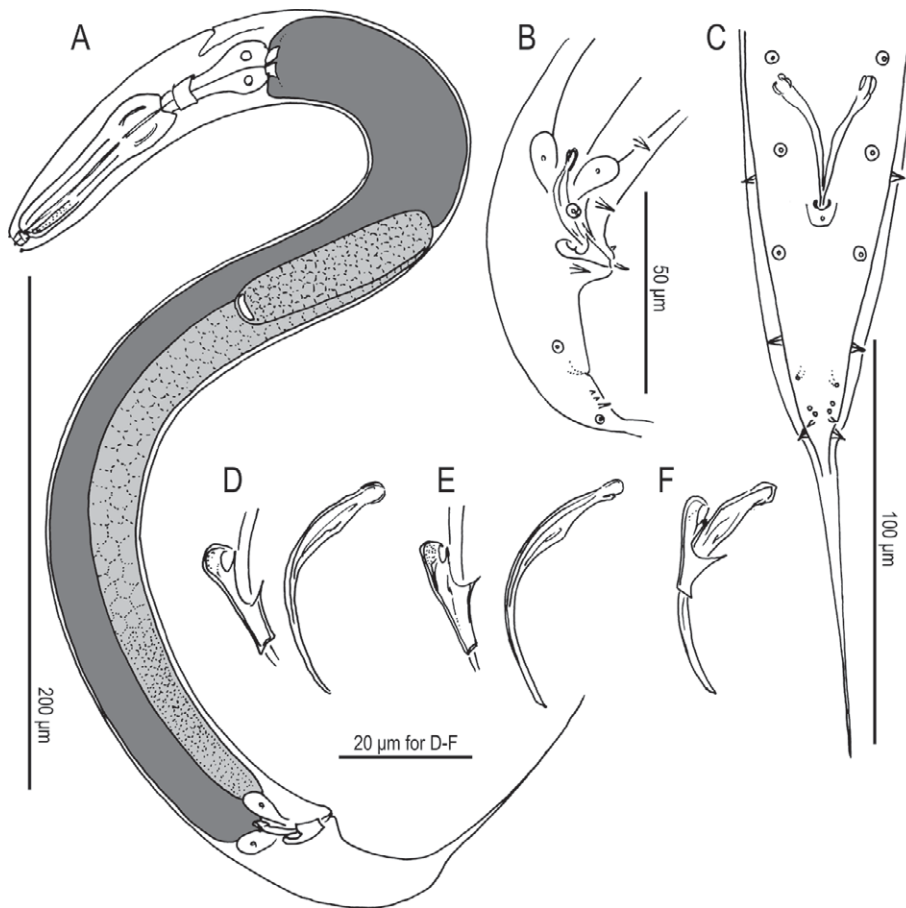
slightly thickened compared to ventral side.

**Eury stomatous form.** Cheilostom divided into six distinctive per- and interradiial plates (Figs. 2E–G, 4F, H). Anterior

papilliform cephalic papillae present in males, as typical for diplogastrid nematodes (Figs. 2A, 4C). Amphidial apertures located at level of posterior end of cheilostomatal plates (Figs. 2A, 4C). Stomatal dimorphism present, with stenostomatous (narrow mouthed) and eury stomatous (wide mouthed) forms occurring in both males and females. Detailed stomatal morphology is described for each species below. Dorsal pharyngeal gland clearly observed, penetrating dorsal tooth to gland opening (Figs. 2D–G, 4E–H). 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. (Figs. 1C, 5A). 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 at level of isthmus to pharyngo-intestinal junction, excretory duct extending anteriorly and reflexed back to position of pore (Figs. 1C, 5A). Deirid observed laterally, located from slightly anterior to slightly posterior of pharyngo-intestinal junction. Hemizonid not clearly observed. Pores, namely "postdeirids" and "predeirids," present and observed laterally (Figs. 1D, 5E), with positions inconsistent among individuals, numbering 5–8 for males and 9–13 for females.

**Stenostomatous form.** Cheilostom consisting of six per- and interradiial plates (Figs. 2B–D, 4E, G). Incision between plates not easily distinguished by light microscopic observation. Anterior end of each plate rounded and elongated to project from stomatal opening and form small flap. Gymnostom short, cuticular ring-like anterior end overlapping cheilostom internally. Dorsal gymnostomatal wall





**Fig. 3.** Male adult of *Pristionchus maxplancki* n. sp. Drawings are of live specimens (non-types) from temporary mounts. **(A)** Whole body of stenostomatous individual, left lateral view. **(B)** Tail region, right lateral view. **(C)** Tail region, ventral view. **(D–F)** Variants of gubernaculum and spicule, right lateral view.

end of each plate rounded and elongated to project from stomatal opening, forming small flap.

**Male.** Ventrally arcuate, strongly ventrally curved at tail region when killed by heat. Testis single, ventrally located, anterior part reflexed to right side (Figs. 3A, 4A). *Vas deferens* not clearly separated from other parts of gonad. Three (two subventral and one dorsal) cloacal gland cells observed at distal end of testis and intestine (Figs. 3B, 5G, I). 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 (Figs. 3C, 5I). Spicule in lateral view smoothly ventrally arcuate, giving spicule about 100° curvature, oval manubrium present at anterior end, lamina/calomus complex clearly expanded just posterior to manubrium, then smoothly tapering to pointed distal end (Figs. 3D–F, 5H). Thick cuticle around tail region, falsely appearing as a narrow leptoderan bursa in ventral view (Fig. 6D, E). Cloacal opening slit-like in ventral view (Figs. 3C, 5I). One small, ventral, single genital papilla on anterior cloacal lip (Figs. 3B, C, 5G, I). Nine pairs of genital papillae and a pair of phasmids present. 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 (Figs. 3B, C, 5G, I). P6 and P7 papilliform and borne from socket-like base, P8 simple or typical thorn-like in shape. Tip of P7 papillae split into two small papilla-like projections. Detailed arrangement of paired papillae and phasmids is described for individual species below. Tail conical, with long spike, about two to three cloacal body diam. long (Figs. 3A, C, 4A, 5G). Bursa or bursal flap absent.

**Female.** Relaxed or slightly ventrally arcuate when killed by heat. Gonad didelphic, amphidelphic (Figs. 1B, 5B). 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 (Figs. 1A, B, 4A, 5B). *Receptaculum seminis* not observed. Vaginal glands present but obscure (Fig. 1G). Vagina perpendicular to body surface, surrounded by sclerotized tissue (Figs. 1B, 5B). Vulva slightly protuberant in lateral view, pore-like in ventral view. Rectum about one anal body diam. long, intestine/rectum junction surrounded by well-

developed sphincter muscle. Three anal glands (two subventral and one dorsal) present but not obvious (Figs. 1E, 5D). Anus in form of dome-shaped slit, posterior anal lip slightly protuberant (Figs. 1F, 5C). Phasmid about one to two anal body diam. posterior to anus (Figs. 1E, 5D). Tail long, smoothly tapered, distal end variable from filiform to long and conical (Figs. 1A, E, 4B, 5D).

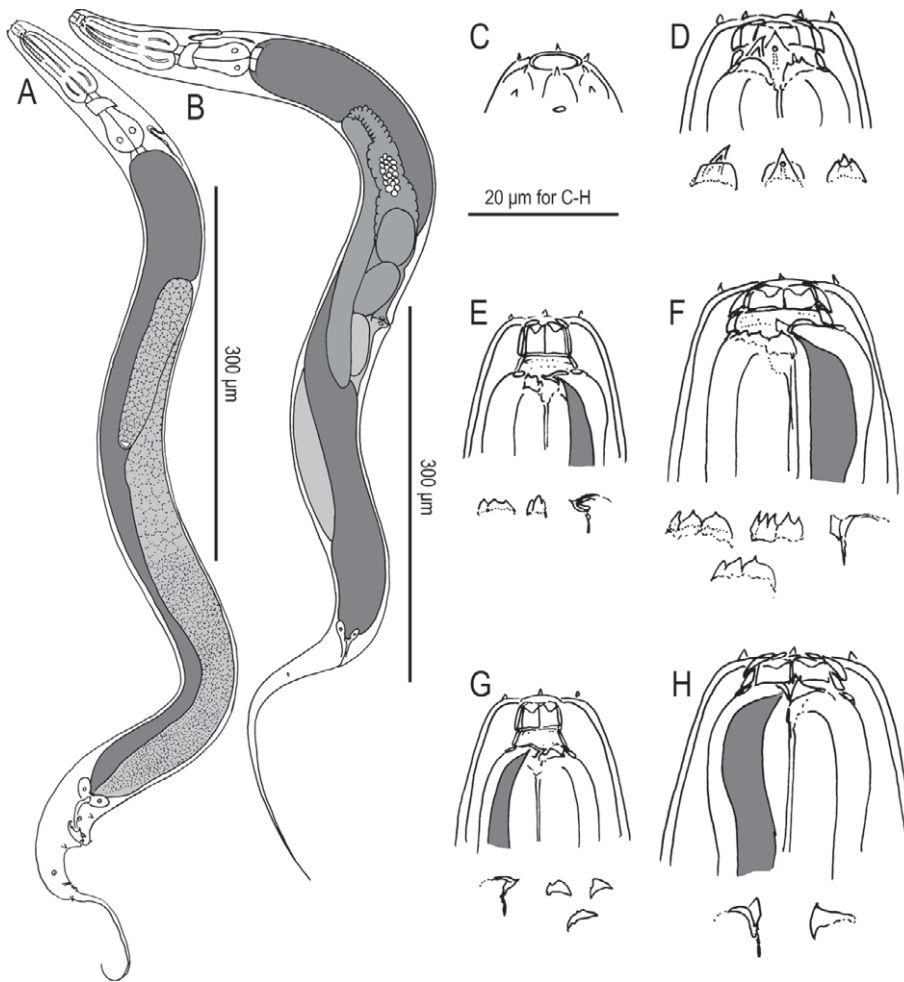
#### Species descriptions based on species-specific characters

##### *Pristionchus maxplancki* n. sp.

(Figs. 1–4, 6)

**Measurements.** See Table 1.

**Description.** *Stenostomatous* form. Stegostom bearing: conspicuous and movable triangular or flint-shaped dorsal tooth (Figs. 2B–D, 6A), often with rough posterior base or denticle-like projection just posterior to base (Fig. 2C); pointed left subventral ridge with minute adventitious denticles on distal side of ridge (i.e., away from body axis) (Figs. 2B, C, 6A, B); pointed right subventral ridge, often with distinct distal adventitious denticle (Fig. 2B–D). Dorsal tooth with strongly sclerotized surface.



**Fig. 4.** *Pristionchus quartusdecimus* n. sp. Drawings are of live specimens (non-types) from temporary mounts. **(A)** Whole body of adult stenostomatous male. **(B)** Whole body of stenostomatous female, right lateral view. **(C)** Lip region of stenostomatous male. **(D)** Stomatal region of eurystomatous female, ventral view; below are right subventral tooth, dorsal tooth, and left subventral denticles. **(E)** Stomatal region of stenostomatous female, left lateral view; below are two variants of left subventral ridge (left) and dorsal tooth (right). **(F)** Stomatal region of eurystomatous female, left lateral view; below are three variants of left subventral ridge (left) and dorsal tooth (right). **(G)** Stomatal region of stenostomatous female, right lateral view; below are dorsal tooth (left) and three variants of right subventral ridge. **(H)** Stomatal region of eurystomatous female, left lateral view; below are dorsal tooth and right subventral tooth.

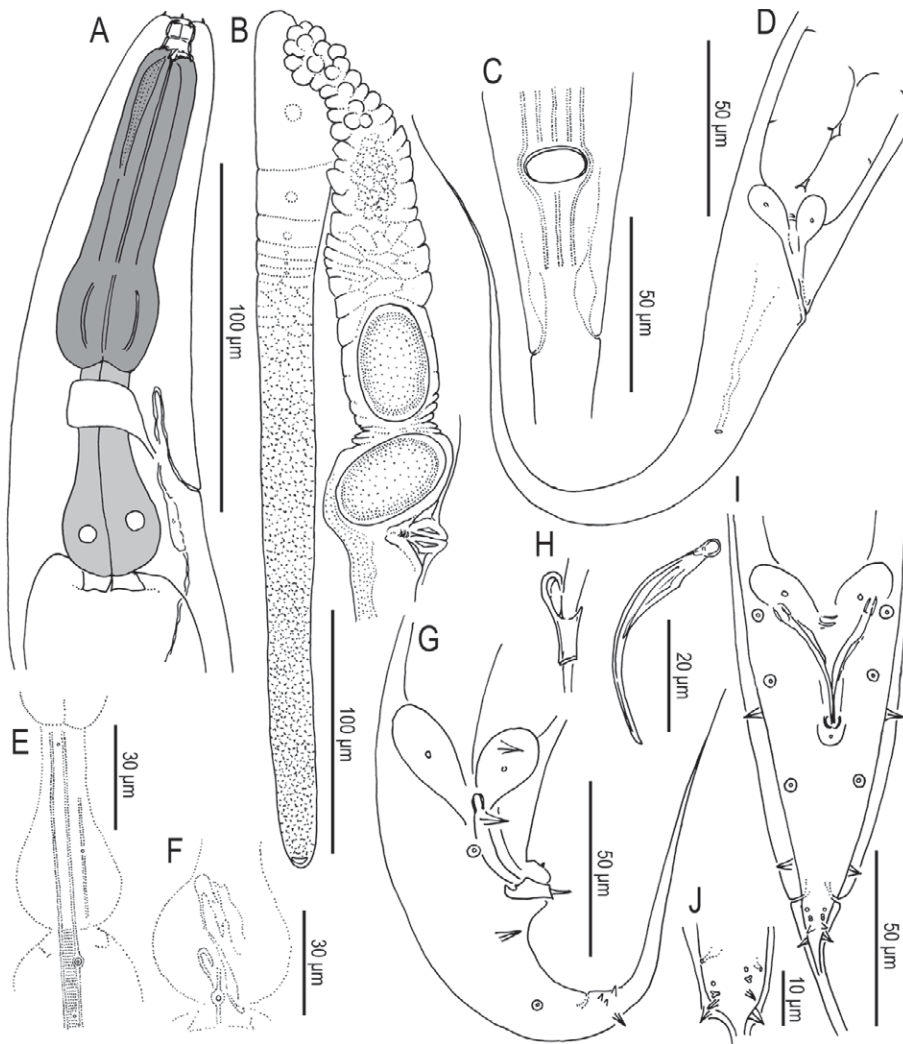
**Eurystomatous form.** Gymnostom with thick cuticle, forming a short, ring-like tube being thicker posteriorly. Anterior end of gymnostom internally overlapping posterior end of cheilostomatal plates. Stegostom bearing: large claw-like dorsal tooth (Fig. 2E–G); large claw-like right subventral tooth (Fig. 2G); ridge of left subventral denticles or cusps of varying numbers and size, i.e., three large cusps, the most lateral of which being sometimes split at tip, or four medium-sized denticles (Fig. 2E, F). Dorsal and right subventral teeth movable. No movement observed for left subventral denticles.

**Male.** Spermatogonia arranged in three to five rows in reflexed part, then well-developed spermatocytes arranged as three to four rows in anterior two-thirds of main branch, then mature amoeboid spermatids arranged in multiple rows in remaining, proximal part of gonad (Fig. 3A). Gubernacu-

lum conspicuous, about one-third of spicule length, broad anteriorly such that dorsal wall is slightly recurved and that dorsal and ventral walls separate at 50–60° angle at posterior end (Figs. 3D–F, 6C). Dorsal side of gubernaculum possessing single, membranous, anteriorly directed process and lateral pair of more sclerotized, anteriorly and obliquely ventrally directed processes (Fig. 3D–F). In lateral view, anterior half of gubernaculum with two serial curves separated by anteriorly and obliquely ventrally directed process, with anterior terminal curvature highly concave and almost closed, and with deep posterior curvature being one-third of gubernaculum length; posterior half forming tube-like process enveloping spicules (Fig. 3D–F). Nine pairs of genital papillae arranged as <P1, P2, P3d, C, P4, P5d, Ph, (P6, P7, P8), P9d> (= <v1, v2, v3d, C, v4, ad, Ph, (v5, v6, v7), pd> in nomenclature of Sudhaus and Fürst von Lieven, 2003), whereby P1, P2, and P4 are equidistant from each other, P2 close to P3d, phasmid (Ph) mid-way between P5d and P6, and P9d clearly posterior to P8 (Fig. 3B, C).

**Female.** Oocytes mostly arranged in three to four rows in distal two-thirds of ovary and in double or single row in rest of ovary, distal tips of each ovary reaching oviduct of opposite gonad branch (Fig. 1B). Anterior part of ovary proximal to flexure a columnella-like structure with about four or five rows of cells (Fig. 1B). Middle part of oviduct serving as spermatheca (Fig. 1B). Eggs in single to multiple-cell stage or even further developed at posterior part of oviduct (= uterus), which in young hermaphrodites being composed of squared or angular cells, long enough to contain one well-developed oocyte (Fig. 1A, B).

**Diagnosis.** Besides its generic characters, *P. maxplancki* is diagnosed by its male papillae arrangement, as described above, and by its stomatal morphology, in which the eurystomatous form has three large left subventral denticles, the most lateral of which is sometimes split, and in which the stenostomatous form shows distally positioned adventitious denticles on both subventral ridges. *Pristionchus maxplancki* is distinguished from other *Pristionchus* species by a stenostomatous form usually with three distal adventitious denticles on the left subventral ridge and one on the right subventral ridge vs. no distal adventitious den-



**Fig. 5.** *Pristionchus quartusdecimus* n. sp. Drawings are of live specimens (non-types) from temporary mounts. (A) Neck region of stenostomatous female, right lateral view. (B) Anterior reproductive tract of female, right lateral view. (C) Anus of female, right lateral view. (D) Tail region of female, right lateral view. (E) Deirid and "postdeirid" and "predeirid" pores, left lateral view. (F) Excretory pore, ventral view. (G) Tail region of male, right lateral view. (H) Gubernaculum and spicule, right lateral view. (I) Tail region of female, ventral view. (J) Variant arrangement of phasmid and male genital papillae P6-P9d.

ticles on either subventral ridge. The species is further distinguished from all other valid *Pristionchus* species where male papilla arrangement is known, except for *P. japonicus*, *P. quartusdecimus*, *P. aerivorus* (Cobb in Merrill and Ford, 1916) Chitwood, 1937 and *P. pseud aerivorus* Herrmann, Mayer, and Sommer, 2006, by <P2, P3d> vs. <P2d, P3> or <(P2, P3d)> (i.e., where both papillae are at same level). It is distinguished from *P. japonicus*, *P. quartusdecimus*, and *P. aerivorus* by P2 and P3d being close vs. clearly apart. *Pristionchus maxplancki* is distinguished from *P. arcanus*, *P. exspectatus*, and *P. pacificus* by P9d being clearly posterior to vs. overlapping P6–P8. The species is distinguished from all other members of the *P. pacificus* species complex, namely *P. arcanus*, *P. exspectatus*, *P. pacificus*, and *P. japonicus*, as well as from *P. quartusdecimus*, by a gubernaculum that is wide anteriorly, such that the dorsal and ventral walls separate at a 60° angle vs. being relatively nar-

row anteriorly, such that walls separate at a 30° angle. *Pristionchus maxplancki* is distinguished from *P. japonicus* by reproductive isolation, namely the inability to produce viable F1 offspring in hybrid crosses (see below). The species is distinguished from all other *Pristionchus* species by its unique SSU rRNA sequence, an 830-bp fragment of which differs from all members of the *P. pacificus* complex by at least five nucleotides (not shown). Finally, *P. maxplancki* is distinguished from *P. pacificus* and phylogenetically more distant hermaphroditic *Pristionchus* species by a gonochoristic reproductive mode.

**Type host and locality.** The culture from which the type specimens were obtained was originally isolated from the body of an adult *Lucanus maculifemoratus* Motschulsky, 1861 (Coleoptera: Lucanidae) collected by N. Kanzaki and M. Herrmann in an oak forest in Fuzawa, Tadami, Fukushima, Japan (37.3542°N, 139.8428°E) in June 2012.

**Type material.** Holotype stenostomatous male, four paratype stenostomatous males, three paratype stenostomatous females, and three paratype eurystomatous females deposited in the University of California Riverside Nematode Collection (UCRNC), Riverside, CA, USA. Two paratype stenostomatous males, one paratype stenostomatous female, and one paratype eurystomatous female (SMNH Type-8423–8425) deposited in the Swedish Natural History

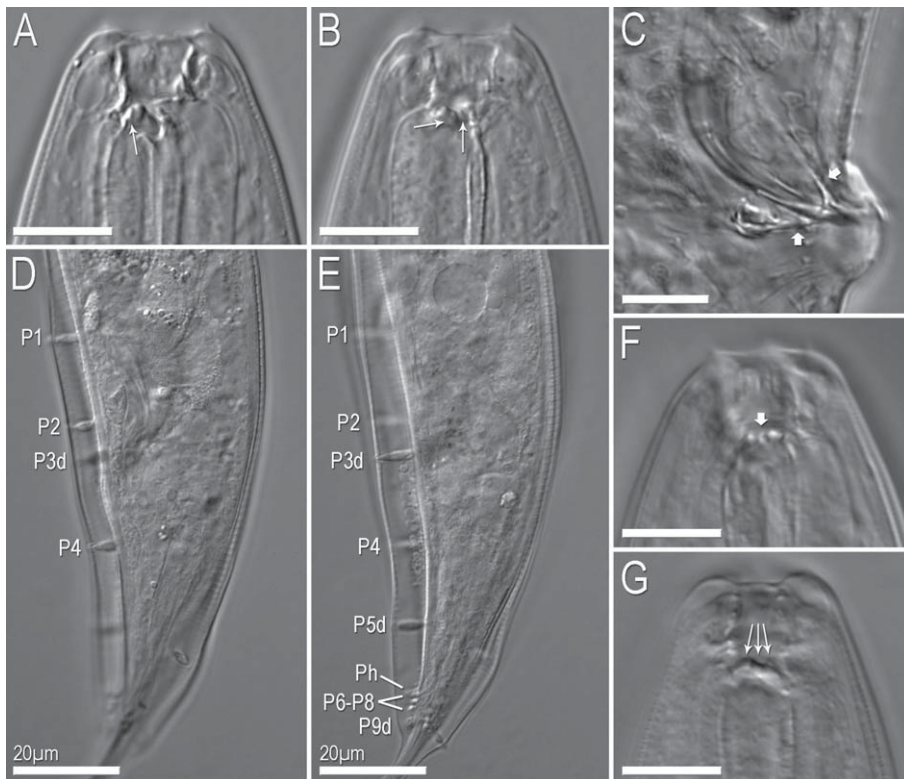
Museum, Stockholm, Sweden. Two paratype stenostomatous males, one paratype stenostomatous female, and one paratype eurystomatous female deposited in the Natural History Museum Karlsruhe, Germany.

**Type strain culture.** Available as living cultures and frozen stocks under culture code RS5594 in the Department of Evolutionary Biology, Max Planck Institute (MPI) for Developmental Biology, Tübingen, Germany, and can be provided to other researchers upon request.

**Etymology.** The specific name, a masculine noun in the genitive case, commemorates the German physicist Max Planck, the honorary namesake of the Society that sponsored this work.

***Pristionchus quartusdecimus* n. sp.**  
(Figs. 4–6)





**Fig. 6.** Nomarski micrographs of diagnostic characters in *Pristionchus maxplancki* n. sp. and *P. quartusdecimus* n. sp. Images are of live specimens (non-types) from temporary mounts. **(A, B)** Stomatal region of a single stenostomatous female of *P. maxplancki* in different focal planes, left lateral view. Focal plane in **(B)** is lateral of that in **(A)**. The left subventral ridge is characterized by adventitious denticles (arrows). Arrows indicate denticles on the left subventral ridge. **(C)** Gubernaculum of *P. maxplancki*. Dorsal and ventral walls (arrows) open at a wide ( $60^\circ$ ) angle. **(D, E)** Male genital papillae of a single individual of *P. quartusdecimus* in different focal planes, oblique left ventral view. Focal plane in **(E)** is dorsal of that in **(D)**. P2 is closer to P1 than to P4; P9d is clearly posterior to P6–P8. **(F)** Stomatal region of stenostomatous female of *P. quartusdecimus*, right lateral view. Right subventral ridge (arrow) is characterized by irregular minute denticles. **(G)** Stomatal region of stenostomatous female of *P. quartusdecimus*, left lateral view. Left subventral ridge is characterized by three peaks (arrows).

Syn. *Pristionchus* sp. 14 *apud* Herrmann et al. (2007), Mayer et al. (2007, 2009), and Kanzaki et al. (2012a, 2012c).

**Measurements.** See Table 2.

**Description.** *Stenostomatous form.* Stegostom bearing: conspicuous and movable triangular or flint-shaped tooth (Fig. 4E, G); right subventral ridge rough, with one to three small peaks of variable shape and size (Figs. 4G, 6F); and left subventral ridge, often wide in transverse plane, with three distinct peaks of variable size (Figs. 4E, 6G). Dorsal tooth with strongly sclerotized surface.

*Eurystomatous form.* Cheilostom and gymnostom of similar height and, together, sometimes two or three times wider than deep (Fig. 4D, F, H). Gymnostom with thick cuticle and thickest posteriorly, forming a short, ring-like tube. Anterior end of gymnostom strongly narrowing at anterior end, internally overlapping posterior end of cheilostomatal plates. Stegostom bearing: large claw-like dorsal tooth (Fig. 4D, F, H); large claw-like right subventral tooth (Fig. 4D, H); often broad left subventral ridge bearing row of either three broad-based denticles or several medium-sized denticles

supported by three broad bases, denticles in both cases often appearing split between bases (Fig. 4F). In ventral view, dorsal and right subventral teeth have anteriorly squared bases (Fig. 4D). Dorsal and right subventral teeth movable. No movement observed for left subventral denticles.

**Male.** Spermatogonia arranged in four or five rows in reflexed part, then well-developed spermatocytes arranged as four to five rows in anterior two-thirds of main branch, then mature amoeboid spermatids arranged in many (e.g., eight to 10) rows in remaining, proximal part of gonad (Fig. 4A). Gubernaculum conspicuous, about or less than one-third of spicule length, only slightly broader anteriorly and with slightly recurved dorsal wall such that dorsal and ventral walls separate at  $30^\circ$  angle at posterior end (Fig. 5H). Dorsal side of gubernaculum possessing single, membranous, anteriorly directed process and lateral pair of more sclerotized, anteriorly directed processes. In lateral view, anterior half of gubernaculum with two serial curves separated by anteriorly directed process, with anterior terminal curvature highly concave and almost closed, and with deep posterior curvature of one-third of gubernaculum length; posterior half forming tube-like process enveloping spicules. Nine pairs of genital papillae arranged

as <P1, P2, (P3d, C), P4, P5d, Ph, (P6, P7, P8), P9d> (= <v1, v2, (v3d, C), v4, ad, Ph, (v5, v6, v7), pd> in nomenclature of Sudhaus and Fürst von Lieven (2003), whereby P2 is closer to P1 than to P4, P2 and P3d clearly apart, phasmid (Ph) closer to P6 than to P5d, and P9d clearly posterior to P8 (Figs. 5G, I, J, 6D, E).

**Female.** Oocytes mostly arranged in five to eight rows in distal two-thirds of ovary and in double or single row in rest of ovary, distal tips of each ovary reaching oviduct of opposite gonad branch. Anterior part of ovary proximal to flexure being columnella-like structure with about four or five rows of cells (Fig. 5B). Middle part of oviduct serving as spermatheca (Fig. 5B). Eggs in single to multiple-cell stage or even further developed at posterior part of oviduct (= uterus), which in young hermaphrodites is composed of squared or angular cells, long enough to contain several eggs in series (Figs. 4B, 5B).

**Diagnosis.** Besides its generic characters, *P. quartusdecimus* is diagnosed by its male papillae arrangement, as described above. It is distinguished from all other species of *Pristionchus* by its stomatal morphology, of which the eurys-



**Table 1.** Morphometrics of stenostomatous male holotype (in glycerin) and male and female specimens of *Pristionchus maxplancki* n. sp. (temporary water mounts). All measurements made in  $\mu\text{m}$  and given 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	—	13	7	10	10
L	673	799 $\pm$ 87 (640–924)	632 $\pm$ 38 (561–664)	1064 $\pm$ 205 (780–1326)	903 $\pm$ 105 (807–1124)
L'	543	656 $\pm$ 76 (535–763)	515 $\pm$ 35 (446–551)	856 $\pm$ 173 (631–1085)	718 $\pm$ 87 (639–888)
a	19	17 $\pm$ 1.7 (13–19)	18 $\pm$ 1.3 (17–20)	14 $\pm$ 1.5 (12–17)	15 $\pm$ 1.8 (12–17)
b	5.9	6.4 $\pm$ 0.7 (5.2–7.5)	4.9 $\pm$ 0.3 (4.3–5.3)	7.4 $\pm$ 0.9 (6.3–8.7)	6.8 $\pm$ 0.5 (5.8–7.8)
c	5.2	5.6 $\pm$ 0.4 (4.7–6.3)	5.4 $\pm$ 0.4 (4.9–6.0)	5.1 $\pm$ 0.4 (4.7–5.8)	4.9 $\pm$ 0.4 (4.0–5.5)
c'	5.7	4.8 $\pm$ 0.5 (4.2–5.9)	4.9 $\pm$ 0.2 (4.5–5.0)	7.3 $\pm$ 0.6 (6.0–8.0)	7.1 $\pm$ 1.1 (5.6–9.3)
T or V	56	56 $\pm$ 5.6 (48–64)	50 $\pm$ 1.8 (48–53)	48 $\pm$ 3.3 (44–55)	47 $\pm$ 1.6 (44–49)
Maximum body diam.	36	49 $\pm$ 9.3 (38–67)	35 $\pm$ 2.3 (31–38)	78 $\pm$ 20 (47–108)	61 $\pm$ 13 (50–79)
Stoma width	4.3	4.8 $\pm$ 0.7 (3.3–5.8)	8.6 $\pm$ 0.8 (7.4–10.0)	5.8 $\pm$ 0.6 (4.7–6.6)	8.8 $\pm$ 1.0 (7.4–10.8)
Stoma height (cheilo- + gymnostom)	6.4	6.2 $\pm$ 0.6 (5.0–7.1)	5.7 $\pm$ 0.7 (4.4–6.6)	7.4 $\pm$ 0.6 (6.3–8.2)	6.6 $\pm$ 0.6 (5.4–7.5)
Stoma height (to base of stegostom)	9.4	9.8 $\pm$ 0.8 (8.4–11.4)	9.9 $\pm$ 0.7 (8.6–10.5)	11.8 $\pm$ 1.1 (10.0–13.2)	11.1 $\pm$ 1.0 (9.2–12.6)
Neck length (head to base of pharynx)	114	125 $\pm$ 6.2 (117–135)	129 $\pm$ 3.0 (125–134)	142 $\pm$ 13 (122–162)	132 $\pm$ 8.2 (119–144)
Anterior pharynx (pro- + metacarpus)	63	70 $\pm$ 3.9 (64–78)	72 $\pm$ 3.7 (67–79)	84 $\pm$ 7.6 (73–97)	78 $\pm$ 4.8 (70–85)
Posterior pharynx (isthmus + basal bulb)	42	48 $\pm$ 3.8 (42–53)	52 $\pm$ 2.1 (48–53)	50 $\pm$ 5.4 (41–57)	47 $\pm$ 3.5 (43–53)
Post./ant. pharynx ratio	67	69 $\pm$ 6.3 (58–78)	72 $\pm$ 5.3 (62–78)	59 $\pm$ 2.9 (55–65)	61 $\pm$ 2.7 (57–66)
Excretory pore from ant. end	99	115 $\pm$ 11 (94–135)	110 $\pm$ 12 (93–123)	132 $\pm$ 16 (103–154)	122 $\pm$ 12 (105–139)
Testis length	375	448 $\pm$ 90 (306–583)	315 $\pm$ 24 (270–339)	—	—
Ant. female gonad length	—	—	—	247 $\pm$ 65 (137–312)	195 $\pm$ 44 (150–287)
Post. female gonad length	—	—	—	214 $\pm$ 60 (112–318)	149 $\pm$ 27 (118–189)
Vulva to anus distace	—	—	—	364 $\pm$ 83 (237–488)	296 $\pm$ 41 (247–364)
Cloacal or anal body diam.	23	30 $\pm$ 3.1 (25–35)	24 $\pm$ 2.1 (21–26)	29 $\pm$ 3.8 (25–45)	26 $\pm$ 2.6 (23–30)
Tail length	130	143 $\pm$ 17 (105–165)	117 $\pm$ 7.9 (105–130)	208 $\pm$ 37 (149–271)	184 $\pm$ 27 (156–236)
Spicule length (curve)	43	42 $\pm$ 2.3 (38–47)	38 $\pm$ 1.4 (35–39)	—	—
Spicule length (chord)	34	34 $\pm$ 2.5 (31–41)	30 $\pm$ 1.4 (29–33)	—	—
Gubernaculum length	14	16 $\pm$ 1.0 (13–17)	13 $\pm$ 1.1 (12–15)	—	—

tomatous form has a row of three broad-based denticles with onion-shaped cusps or several medium-sized denticles supported by three broad bases and the stenostomatous

form has a left subventral ridge with three distinct peaks of variable size and a right subventral ridge with one or two small peaks of variable shape. The species is further distinguished from all other valid *Pristionchus* species where the male papillae arrangement is known, except for *P. japonicus* and *P. aerivorus*, by <P2, P3d>, whereby they are clearly separated vs. <P2d, P3>, <(P2, P3d)>, or <P2d, P3>. *Pristionchus quartus-decimus* is further distinguished from *P. japonicus* by having no adventitious denticles vs. one adventitious denticle on the left subventral ridge of the eurystomatous form. It is further distinguished from *P. maxplancki* by having no vs. several adventitious denticles on the subventral ridges of the stenostomatous form and by an anteriorly narrowed gubenaculum, such that the dorsal and ventral walls separate at a 30° vs. 60° angle. It is distinguished from *P. maxplancki*, *P. arcanus*, *P. exspectatus*, and *P. pacificus* by: P2 being closer to P1 than to P4 vs. P2 being closer to P4 than to P1 or being equidistant from P1 and P4; P9d being clearly posterior to vs. overlapping P6–P8. Finally, the species is distinguished from all other *Pristionchus* species by its unique SSU rRNA sequence.

#### Type host and locality.

The culture from which the type specimens were obtained was originally isolated from the body of an adult of *Exomala orientalis* (Waterhouse, 1875; Piattella and Sabatinelli, 1994) (Coleoptera: Scarabaeidae) collected by M. Herrmann at the Mokawa River in Amagasaki-shi, Hyogo,

Japan in June 2006.

**Type material.** Holotype stenostomatous male, four paratype stenostomatous males, three paratype stenostom-

**Table 2.** Morphometrics of stenostomatous male holotype (in glycerin) and male and female specimens of *Pristionchus quartusdecimus* n. sp. (temporary water mounts). All measurements made in  $\mu\text{m}$  and given 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	–	15	5	10	10
L	869	732 $\pm$ 59 (625–820)	586 $\pm$ 31 (549–619)	921 $\pm$ 141 (747–1250)	778 $\pm$ 34 (719–839)
L'	716	623 $\pm$ 54 (542–726)	480 $\pm$ 38 (436–521)	743 $\pm$ 109 (605–970)	615 $\pm$ 18 (590–651)
a	19	14 $\pm$ 1.3 (12–17)	15 $\pm$ 1.4 (12–16)	13 $\pm$ 2.2 (11–17)	16 $\pm$ 1.6 (12–18)
b	6.8	5.4 $\pm$ 0.3 (4.9–6.4)	4.8 $\pm$ 0.2 (4.6–5.1)	6.0 $\pm$ 0.5 (5.6–7.3)	5.5 $\pm$ 0.2 (5.1–5.9)
c	5.7	6.9 $\pm$ 1.0 (5.1–8.7)	5.6 $\pm$ 0.7 (4.8–6.6)	5.3 $\pm$ 0.7 (4.2–6.7)	4.8 $\pm$ 0.4 (4.2–5.5)
c'	6.4	3.6 $\pm$ 0.6 (3.0–4.9)	3.3 $\pm$ 0.2 (3.0–3.5)	5.9 $\pm$ 0.9 (4.4–7.2)	7.4 $\pm$ 0.6 (6.4–8.3)
T or V	56	52 $\pm$ 3.6 (45–58)	37 $\pm$ 3.5 (31–40)	47 $\pm$ 2.8 (40–50)	47 $\pm$ 1.0 (45–49)
Maximum body diam.	47	30 $\pm$ 2.3 (27–36)	41 $\pm$ 5.8 (35–50)	71 $\pm$ 14 (45–87)	50 $\pm$ 6.9 (42–63)
Stoma width	4.7	5.4 $\pm$ 0.3 (4.9–6.3)	8.9 $\pm$ 0.02 (8.8–8.9)	5.7 $\pm$ 0.8 (4.4–7.0)	9.5 $\pm$ 0.6 (8.5–10.8)
Stoma height (cheilo- + gymnostom)	7.2	6.5 $\pm$ 0.8 (5.1–7.9)	4.8 $\pm$ 0.1 (4.7–4.8)	7.9 $\pm$ 1.0 (7.0–10.3)	5.7 $\pm$ 0.6 (4.7–7.0)
Stoma height (to base of stegostom)	9.9	8.6 $\pm$ 0.9 (6.9–10.0)	5.8 $\pm$ 0.1 (5.6–5.9)	10.5 $\pm$ 1.1 (8.6–13.0)	8.7 $\pm$ 0.8 (7.5–10.0)
Neck length (head to base of pharynx)	127	135 $\pm$ 7.5 (120–146)	121 $\pm$ 3.1 (116–125)	154 $\pm$ 13 (133–172)	141 $\pm$ 4.7 (135–149)
Anterior pharynx (pro- + metacarpus)	72	80 $\pm$ 4.5 (72–86)	72 $\pm$ 3.2 (67–75)	91 $\pm$ 7.0 (77–101)	81 $\pm$ 2.4 (76–84)
Posterior pharynx (isthmus + basal bulb)	48	48 $\pm$ 3.0 (42–52)	45 $\pm$ 0.6 (44–45)	55 $\pm$ 6.4 (43–61)	54 $\pm$ 3.7 (50–61)
Post./ant. pharynx ratio	67	60 $\pm$ 1.9 (58–78)	62 $\pm$ 3.1 (59–67)	60 $\pm$ 4.0 (50–64)	67 $\pm$ 4.7 (60–74)
Excretory pore from ant. end	132	108 $\pm$ 7.9 (95–122)	95 $\pm$ 4.6 (89–101)	123 $\pm$ 11 (106–143)	114 $\pm$ 3.5 (109–122)
Testis length	488	383 $\pm$ 47 (298–473)	215 $\pm$ 16 (193–239)	–	–
Ant. female gonad length	–	–	–	190 $\pm$ 47 (133–172)	144 $\pm$ 13 (120–161)
Post. female gonad length	–	–	–	123 $\pm$ 11 (106–143)	142 $\pm$ 14 (120–166)
Vulva to anus distace	–	–	–	318 $\pm$ 49 (276–437)	258 $\pm$ 11 (235–273)
Cloacal or anal body diam.	24	30 $\pm$ 2.3 (27–36)	32 $\pm$ 2.9 (28–35)	30 $\pm$ 4.3 (24–40)	22 $\pm$ 2.2 (19–26)
Tail length	153	109 $\pm$ 19 (81–143)	106 $\pm$ 8.4 (93–114)	178 $\pm$ 42 (139–281)	163 $\pm$ 18 (130–191)
Spicule length (curve)	46	44 $\pm$ 2.9 (38–51)	37 $\pm$ 0.5 (36–38)	–	–
Spicule length (chord)	38	36 $\pm$ 2.1 (32–42)	33 $\pm$ 0.7 (32–34)	–	–
Gubernaculum length	16	15 $\pm$ 1.1 (13–18)	14 $\pm$ 0.6 (12–16)	–	–

atous females, and three paratype eurystomatous females deposited in the UCRNC, Riverside, CA, USA. Two paratype stenostomatous males, one paratype stenostomatous

female, and one paratype eurystomatous female (SMNH Type-8426–8428) deposited in the Swedish Natural History Museum, Stockholm, Sweden. Two paratype stenostomatous males, one paratype stenostomatous female, and one paratype eurystomatous female deposited in the Natural History Museum Karlsruhe, Germany.

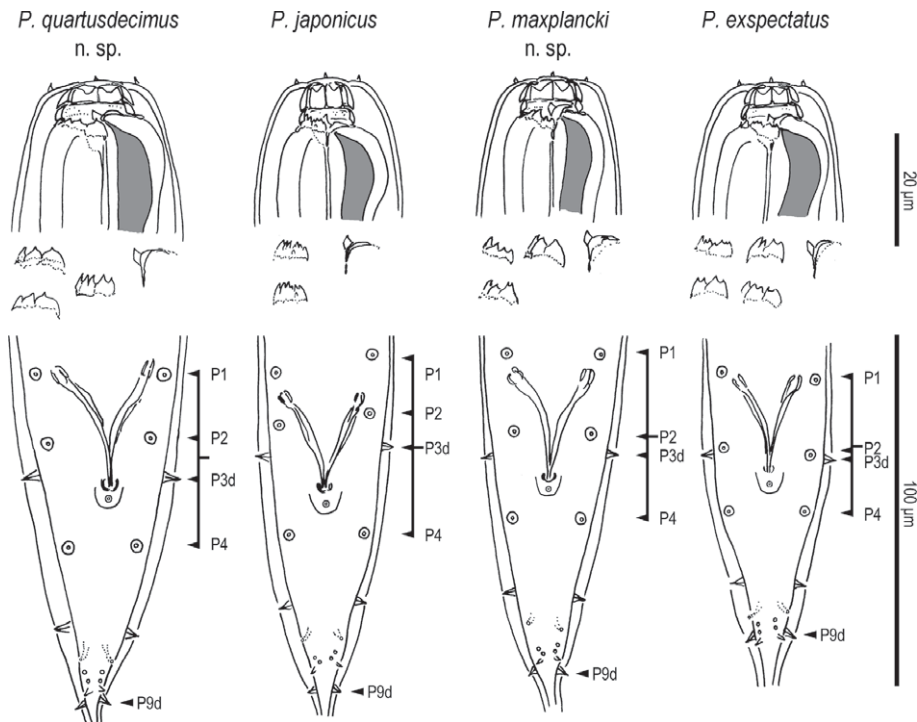
**Type strain culture.** Available as living cultures and frozen stocks under culture code RS5230 in the Department of Evolutionary Biology at the MPI for Developmental Biology and can be provided to other researchers upon request.

**Etymology.** The species epithet is the Latin adjective for “fourteenth” and refers to the previous running identifier of this species.

**Remarks on morphological characters**

Consistent with previous descriptions of the *Pristionchus* species complex (Kanzaki et al., 2012a), morphological diagnosis of species in the group is largely based on stegostomatal morphology and the male papillae arrangement. Stegostomatal morphology is variable within as well as among species, but reporting variation of this morphology reveals consistent differences among species, such as the size, shape, and arrangement of the left subventral denticles in the eurystomatous form (Fig. 7) and the presence of peaks or adventitious denticles in the stenostomatous form (Figs. 2, 4). A comparison of the male tail among all members of the complex and with *P. quartusdecimus* (Fig. 7) shows that the position of P2

relative to P3d, the spacing of P3d between P1 and P4, and the position of P9d relative to P6–P8 to be the most informative characters for diagnosing individual species.



**Fig. 7.** Comparative morphology of the eurystomatous stoma (above) and male tail (below) in *Pristionchus quartusdecimus* n. sp. and three representatives of the *P. pacificus* species complex. Characters are alike in *P. pacificus*, *P. arcanus*, and *P. expectatus* and are thus shown only for the latter species. The clearest stomatal variation in these species is in the left subventral ridge of the stegostome (below left in each mouth diagram). Arrangement of male genital papillae are most variable in the relative positions of P1–P4 and in the placement of P9d relative to P6–P8.

### Mating experiments

Reciprocal mating tests between *P. maxplancki* and *P. japonicus* did not result in any viable F1 progeny, whereas control crosses within each species yielded numerous progeny. Therefore, both species were considered biologically unique.

### Molecular characterization and phylogenetic analysis

All sequences for *P. maxplancki* were original in this study and have been deposited in the GenBank database under the accession numbers KC491680 (SSU rRNA) and KC491681–KC491703 (ribosomal protein genes); sequences for *P. quartusdecimus* were available from GenBank (Mayer et al., 2007). The 830-bp SSU rRNA sequence obtained for *P. maxplancki*, differing from all other species in the *P. pacificus* species complex by at least five nucleotides, clearly diagnose the species as unique and new. Phylogenetic analysis of sequences of the partial SSU rRNA and 25 ribosomal protein loci further revealed divergence of *P. maxplancki* and *P. quartusdecimus* from all other *Pristionchus* species (Figs. 8, 9).

The final alignment comprised 1801 parsimony-informative sites. Trees from both the ML and Bayesian analyses upheld the *Pristionchus* species complex, including *P. japonicus*, *P. maxplancki*, *P. arcanus*, *P. expectatus*, and *P. pacificus* as a robustly supported (100% BS and PP) monophyletic group. Within this group, *P. japonicus* was the most basal and *P. maxplancki* was, among all molecularly

characterized *Pristionchus* species, highly supported (99% BS, 100% PP) as the immediate out-group to a clade of *P. arcanus*, *P. expectatus*, and *P. pacificus*.

A monophyletic clade of *Pristionchus* including the two new species but excluding *P. fissidentatus* Kanzaki et al. (2012c), *P. elegans* Kanzaki et al. (2012c), and *P. bucculentus* Kanzaki et al., 2013 (“sp. RS5596”) was moderately well supported (89% BS, 100% PP). The position of *P. quartusdecimus* differed between the ML and Bayesian analyses and was only poorly supported in either analysis: namely, whereas *P. quartusdecimus* was clearly out-group to the *P. pacificus* species complex, whether it is also the out-group to a more inclusive group of *Pristionchus* species was unresolved.

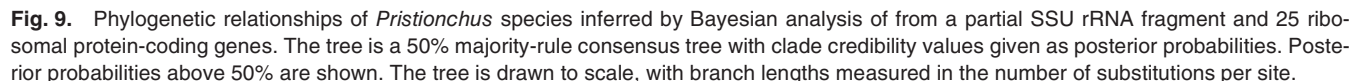
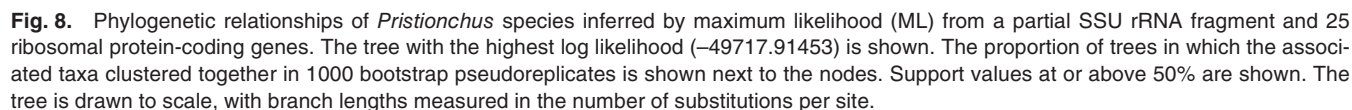
All other relationships, except for the position of *P. fissidentatus*, were consistent with previous analyses (Kanzaki et al., 2012a, b). The position of *P. fissidentatus* was unresolved, being inconsistent between the two analyses and poorly supported in both (< 50% BS, 71% PP).

### DISCUSSION

Identification of a new member of the *P. pacificus* species complex, *P. maxplancki*, as well as a new putative out-group, *P. quartusdecimus*, allows greater precision for reconstructing ancestral states in the complex. Specifically, *P. maxplancki* is herein identified as a closer outgroup to the species triad of *P. pacificus*, *P. arcanus*, and *P. expectatus*. Since the discovery of the latter two species, preliminary work has shown the potential to unravel the evolutionary genetics of traits divergent among these species. Most notable is the possibility to reveal the genetic basis of hermaphroditism in *P. pacificus* (Kanzaki et al., 2012a), such as by hybrid crosses, as performed in a pair *Caenorhabditis* species (Woodruff et al., 2010). Another prospect is interspecific tests of fitness variables that are divergent within *P. pacificus*. The ability to reconstruct pheromonal signaling profiles in nematode species including *P. pacificus* (Bose et al., 2012; Choe et al., 2012) will in principle allow tracking the divergence of conspecific communication across a closely related suite of species. Possibilities for harnessing the comparative model system of *P. pacificus* and its sibling species have yet to mature. Providing denser taxon sampling within this comparative system will increase the detail with which they can be realized.

Increased representation of *Pristionchus* species, including *P. maxplancki* and *P. quartusdecimus*, tests the boundaries to which species can be diagnosed morphologi-





cally. Most traditionally diagnostic characters, including body measurements and ratios, gonad attributes, tail shape, and cuticular surface structures show little to no consistent differences among species of *Pristionchus* (Herrmann et al., 2006b; Kanzaki et al., 2012a, c). The present study confirms that stomatal characters are among the most detectably different among species, although these characters also show variation within species that overlaps with that among species. Therefore, reporting the extent of variability within species will be important for recognizing real differences among *Pristionchus* species as more species become known. For example, adventitious denticles, although individually variable, were consistently observed in stenostomatous individuals of *P. maxplancki*. Likewise, the presence of three peaks on the left subventral ridge of the stenostomatous form was also generally consistent in *P. quartusdecimus*, although the size and shape of these peaks were variable. A more precise understanding of the plasticity of mouthparts and of real differences among species in *Pristionchus* will be afforded by denser taxon representation, possibly through further sampling in Japan.

Male sexual characters, notably gubernaculum shape and the genital papillae arrangement, were also reliable in diagnosing *P. maxplancki* and *P. quartusdecimus* from other *Pristionchus* species. Although the finite variation observed in papillae patterns may limit the extent to which they are unique in a given species, the congruence of these characters with other diagnostic features can separate homology from convergence. For example, the pattern <P2d, P3> characteristic of *P. japonicus*, *P. maxplancki*, and *P. quartusdecimus* is also found in a clade of *P. aerivorus*, *P. pseud aerivorus*, and *P. maupasi* (Potts, 1910) Paramonov, 1952 but not *P. americanus* Herrmann, Mayer, and Sommer (2006), *P. marianneae* Herrmann, Mayer, and Sommer, 2006, or *P. pauli* Herrmann, Mayer, and Sommer, 2006 (Kanzaki et al., in press). Consideration of an independent molecular phylogeny clearly identifies this pattern as convergent evolution. Namely, the transformation series of the arrangement of P1–P4 in *P. quartusdecimus* and the *P. pacificus* species complex (Fig. 7) has revealed intermediate evolutionary steps in the various groups where such characters appear.

The ongoing discovery of new *Pristionchus* species from Japan (Herrmann et al., 2007; Kanzaki et al., 2011, 2012a, c, 2013) underscores the importance of East Asia, and Japan in particular, for the radiation of species in the genus. The cosmopolitan species *P. pacificus* is nested in a clade of species all known so far from Japan (Figs. 8, 9), which with the inclusion of *P. maxplancki* provides further support for an origin of *P. pacificus* in a region including Japan. Although more sampling is necessary to rule out the ranges of these species in other parts of the world, increasingly dense sampling of *Pristionchus* in Europe (Herrmann et al., 2006a; unpubl.), the Americas (Herrmann et al., 2006b), and islands in the Indian Ocean (Herrmann et al., 2010; Kanzaki et al., 2012c) upholds a coherent biogeographic pattern among gonochoristic *Pristionchus* species. Besides the support that *P. maxplancki* gives to a Japanese or East Asian origin of *P. pacificus* and its two closest sibling species, a Japanese locality for *P. quartusdecimus* supports a more ancient origin of *Pristionchus* species in the region. Its posi-

tion as the outgroup to a clade including the *P. pacificus* species complex (Figs. 8, 9) lends support to an origin of the complex in a region including Japan. Greater taxon representation may help to resolve relationships and thus historical biogeography of *P. quartusdecimus* with respect to other *Pristionchus* species, including *Parapristionchus giblindavisi* (Kanzaki et al., 2012a) and *P. elegans* (Kanzaki et al., 2012c), whose known ranges so far support a Japanese or East Asian origin for the genus.

## ACKNOWLEDGMENTS

We thank Heike Haussmann and Gabi Eberhardt for maintaining live cultures and frozen stocks of *Pristionchus maxplancki* (RS5594) and *P. quartusdecimus* (RS5230). We also thank Vladislav Susoy for helpful comments and suggestions.

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(Received December 10, 2012 / Accepted March 12, 2013)