Two new Species of Pristionchus (Nematoda: Diplogastridae) include the Gonochoristic Sister Species of P. fissidentatus

Abstract

The genus Pristionchus (Kreis, 1932) consists of more than 30 soil nematode species that are often found in association with scarab beetles. Three major radiations have resulted in the “maupasi species group” in America, the “pacificus species group” in Asia, and the “lheritieri species group,” which contains species from Europe and Asia. Phylogenetic analysis indicates that a group of three species, including the gonochorists P. elegans and P. bucculentus and the hermaphrodite P. fissidentatus, is basal to the above-mentioned radiations. Two novel species are described here: Pristionchus paulseni sp. n. from Taiwan and P. yamagatae sp. n. from Japan by means of morphology, morphometrics and genome-wide transcriptome sequence analysis. Previous phylotranscriptomic analysis of the complete Pristionchus genus recognized P. paulseni sp. n. as the sister species of P. fissidentatus, and thus its importance for macro-evolutionary studies. Specifically, the gonochorist P. paulseni sp. n. and the hermaphrodite P. fissidentatus form a species pair that is the sister group to all other described Pristionchus species. P. paulseni sp. n. has two distinct mouth forms, supporting the notion that the mouth dimorphism is ancestral in the genus Pristionchus.

Key words

Pristionchus pacificus, P. fissidentatus, Taiwan, Japan, Scarab beetles.

The genus Pristionchus (Kreis, 1932) with the model organism P. pacificus has emerged as an important group of nematodes to study various aspects of macroevolution, including comparative developmental biology, phenotypic plasticity, evolutionary ecology, and comparative genomics (Sommer et al., 1996; Sommer, 2015). Many of these studies critically depend on deep taxon sampling and a robust phylogenetic framework (Susoy et al., 2015). The isolation of new Pristionchus species was fostered by the original discovery that these nematodes are often found in a necromenic and entomophilic association with scarab beetles (Herrmann et al., 2006, 2007; Kanzaki et al., 2012b). Subsequent systematic samplings resulted in the characterization of more than 30 Pristionchus species with four distinct biogeographically restricted species groups (Ragsdale et al., 2015; Herrmann et al., 2016).

The three major radiations include the “maupasi species group” in America, the “pacificus species group” in Asia and the “lheritieri species group,” which contains species from Europe and Asia (Ragsdale et al., 2015). As outgroup to these radiations, the “elegans species group” found in Asia consists of only two gonochorists: P. elegans and P. bucculentus (Kanzaki et al., 2012a; Ragsdale et al., 2013, 2015). However, an additional hermaphroditic species, P. fissidentatus, is an outgroup to the “elegans species group” and is the sister group to all described beetle-associated Pristionchus species. Given that most of the seven hermaphroditic species in the genus have evolved independently of one another, they allow the detailed analysis of parallel evolution, e.g., the influence of mating-type transitions on longevity (Weadick and Sommer, 2016; Rödelsperger et al., 2018). P. fissidentatus is of particular interest, given its phylogenetic position;
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however, it is a hermaphroditic species that has no sister group relationship with any particular species.

Here, we describe two new species. *P. yamagatae* sp. n. was isolated from *Holotricha sp.* (Coleoptera: Melolonthinae) in Japan and belongs to the *Heritieri*-species-complex. *P. paulseni* sp. n. was isolated from Taiwan and represents the gonochoristic sister species of *P. fissidentatus*. This species exhibits a mouth dimorphism found in many *Pristionchus* species, suggesting that mouth-form plasticity is an ancestral character in the genus. Most importantly, *P. paulseni* sp. n. and *P. fissidentatus* form a new species group of the genus that represents the sister group to all described beetle-associated *Pristionchus* species. Thus, the gonochorist *P. paulseni* sp. n. has a crucial position in the phylogeny of *Pristionchus*. In order to characterize both species, morphology, morphometrics, mating experiments and genome-wide transcriptome sequencing are used.

**Materials and methods**

**Nematode cultivation**

One strain of *P. paulseni* sp. n. was isolated from an adult of *Lucanus dybowskii taianus* (Miwa, 1937) (Coleoptera: Lucanidae) collected at Pilu sacred tree, Taroko National Park, Taiwan. The strain of *P. yamagatae* sp. n. was isolated from an adult of *Holotricha sp.* (Hope, 1837) (Coleoptera: Scarabaeidae, Melolonthinae) collected at Yuza, Yamagata, Japan. Both strains have been kept in laboratory culture on NGM agar plates seeded with *Escherichia coli* strain OP50 under the culture code and freezing voucher numbers RS5918 (*P. paulseni* sp. n.) and RS5964 (*P. yamagatae* sp. n.).

**Morphological observation and preparation of type material**

Light microscopic observations for drawings and morphometrics were conducted using live nematode material, which was handpicked from culture plates (Kanzaki et al., 2013).

**Molecular characterization and phylogenetic analysis**

A species phylogeny of the complete *Pristionchus* genus was reconstructed as described in Rödelsperger et al. (2018). In the following, we give a brief summary about the generation of the *Pristionchus* phylogeny (please see Rödelsperger et al., 2018 for full details). For all cultivable *Pristionchus* species, worms were grown on NGM plates seeded with *E. coli* OP50 at 20°C and total RNA was isolated from 2–3 mixed-stage plates per species using standard Trizol extraction following the manufacturers’ instructions (Zymo Research, CA, USA). RNA-seq libraries were prepared using TruSeq RNA library preparation kit v2 (Illumina, Inc., CA, USA), according to the manufacturer’s instructions, from 1 µg of total RNA in each sample and sequenced on the Illumina HiSeq3000 platform, yielding a median of 14m paired reads (2 × 150bp) per species. Raw reads were submitted to the European nucleotide archive under the study accession PRJEB20959. RNA-seq reads were assembled into transcriptomes using Trinity (version v2.2.0) (Grabherr et al., 2011). For further analysis, only the first reported isoform per gene was selected and the longest complete or partial ORF (≥60 amino acids) was called. Orthologous clusters were generated by orthAgogue (Ekseth et al., 2014) and protein sequences were aligned using the MUSCLE software (version 3.8.31) (Edgar, 2004). 2,092 high quality alignments containing at least 14 species (without any duplication), with at least 50 amino acid positions with coverage in all represented species, were concatenated into a supermatrix spanning 350,000 amino acids. On the basis of the previous analysis of dozens of gene families (Baskaran et al., 2015), we chose the LG substitution model to reconstruct a maximum-likelihood tree using RA×ML (version 8.2.9, options –m PROTGAMMAILG –f a –N 100) (Stamatakis, 2014).

**Results**

**Description of common characters**

The majority of *Pristionchus* species are cryptic and typologically similar to each other, including the two novel species described here. In general, *Pristionchus* species are distinguished from each other by stomatal and male tail characters only (for review see Ragsdale et al., 2015). Therefore, the general morphology, e.g., body shape and gonadal structures, is described first as common characters, and then the distinctive characters are described for each species.

**Adult**

Their characteristics include a cylindrical and stout body, a thick cuticle, with fine annulation and clear longitudinal striations. Lateral field consists of two lines, weakly distinguishable from body striation with the presence of deirid. Head exists without apparent lips and with six short and papilliform labial sensilla. Four small, papilliform cephalic papillae are present in
males, as typical for diplogastrid nematodes. Amphidial apertures are located on the lateral sector, slightly dorsally shifted, at level of margin of cheiloo- and gymnostom. Stomatal dimorphism is present, with stenostomatous (narrow mouthed) and eurystomatous (wide mouthed) forms occurring in both males and females, but male eurystomatous form is not as common as females. Detailed stomatal morphology is described below. Dorsal pharyngeal gland is clearly observed, with a penetrating dorsal tooth to gland opening. The anterior part of pharynx (=pro and metacorpus) is 1.5 times as long as the posterior part (=isthmus and basal bulb). Procorpus is very muscular, stout, occupying half to two-thirds of the corresponding body diameter. Metacorpus is very muscular, forming a well-developed median bulb. Isthmus is narrow, not muscular. Basal bulb is glandular. Pharyngoo-intestinal junction is clearly observed; it is well-developed. Nerve ring is usually surrounding the middle region of isthmus. Excretory pore is not conspicuous, ventrally located at the level of isthmus to pharyngo-intestinal junction; excretory duct extends anteriorly and is reflected back to the position of pore. Deirid is observed laterally, located from the level of pharyngo-intestinal junction to one body diameter and posterior to the junction. Hemizonid is not clearly observed. Lateral glands, small pores connected to secretory cell, are present and observed on the lateral body surface, with positions inconsistent among individuals, numbering 5 to 8 for males and 9 to 13 for females. Because the structure is very small and indistinctive, the consistency of their position and number was not confirmed by light microscopy.

Stenostomatous form

Cheilostom consists of six per- and interradial plates. Incision between plates is not easily distinguished by light microscopy. The anterior end of each plate is usually rounded and elongated to project from stomatal opening and forms a small flap. Some more details are described for each species below. The anterior end of each plate sometimes splits into two, forming two narrow flaps, i.e., number of cheilostomatal flaps varies from 6 to 12, regardless of the number of plates (=6). Gymnostom is present with a thick cuticle, forming a short, ring-like tube being thicker posteriorly. The anterior end of gymnostom is internally overlapping the posterior end of cheilostomatal plates. Stegostom is divided into three subsections: pro-meso, meta and telostegostom. Pro-mesostegostom forms a weakly sclerotized cup-like cavity connecting stoma and pharynx.

Eurystomatous form

Cheilostom is divided into six distinctive per- and interradial plates. The anterior part of each plate is usually rounded and elongated to project from stomatal opening, forming a small flap. The anterior part of each plate is sometimes split into two, forming two narrow flaps, i.e., number of cheilostomatal flaps varies from 6 to 12, regardless of the number of plates (=6). Gymnostom is present with a thick cuticle, forming a short, ring-like tube being thicker posteriorly. The anterior end of gymnostom is internally overlapping the posterior end of cheilostomatal plates. Stegostom is divided into three subsections: pro-meso, meta and telostegostom. Pro-mesostegostom forms a weak cuticularized ring surrounding the anterior edge of pharynx. Metastegostom bears large claw-like dorsal tooth, large claw-like right subventral tooth, ridge of left subventral denticles or cusps, varying in number and size. Dorsal and right subventral teeth are movable. No movement is observed for left subventral denticles. Telostegostom forms a weakly sclerotized cup-like cavity connecting stoma and pharynx.

Male

They are ventrally arcuate and become strongly ventrally curved at tail region when killed by heat. Testis is single and ventrally located; the anterior part is reflexed to the right side. Spermatogonia is arranged in three to five rows in the reflexed part; then well-developed spermatocytes are arranged in three to four rows in anterior two-thirds of the main branch; then mature amoeboid spermatids are arranged in multiple rows in remaining, proximal part of gonad. Vas deferens is not clearly separated from other parts of gonad. Three (two subventral and one dorsal) cloacal gland cells are observed at distal ends of testis and intestine. Spicules are paired and separate. Spicules are smoothly curved in ventral view, adjacent to each other for distal third of their length, smoothly tapering to pointed distal end. Spicule in lateral view is smoothly ventrally arcuate, giving spicule about 100° curvature; oval manubrium is present at the anterior end; lamina/calomus complex (blade) is clearly expanded slightly posterior to manubrium (ca
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1/4 of blade length from anterior), smoothly tapering to pointed distal end. Gubernaculum conspicuous, about one-third of spicule length, is broad anteriorly such that dorsal wall is slightly recurved and that dorsal and ventral walls separate at 50 to 60° angle at the posterior end. The dorsal side of gubernaculum possesses a single, membranous, anteriorly directed process and a lateral pair of more sclerotized, anteriorly and obliquely ventrally directed processes. In lateral view, anterior half of gubernaculum is present with two serial curves separated by anteriorly and obliquely ventrally directed process, with anterior terminal curvature being highly concave and almost closed, and with deep posterior curvature being one-third of the gubernaculum length; posterior half forms a tube-like process enveloping spicules. A thick cuticle exists around tail region, falsely appearing as a narrow leptoderan bursa in ventral view. Cloacal opening (co) is slit-like in ventral view. One small, ventral, single genital papilla (vs) exists on anterior cloacal lip. Nine pairs of genital papillae (v1-v7, ad, pd) and a pair of phasmids (ph) are present, with three pre-cloacal and six postcloacal pairs. Within those pairs, three distal ventral pairs (v5, v6, and v7) and a dorsal pair (pd) are close to each other around the posterior end of tail (just anterior to the root of spike). Anterior five pairs of papillae (v1-4 and ad) are present, almost equal in size, rather large and conspicuous; v7 and pd papillae are obviously smaller than anterior five pairs; v5 and v6 are very small, sometimes difficult to observe with light microscope. Anterior two pairs of the ventral triplet papillae (v5 and v6) papilliform are present and born from a socket-like base, v7 is simple or typical thorn-like in shape. The tip of v6 papillae is split into two small papilla-like projections. A detailed arrangement of papillae and phasmid will be observed for each species below. The tail is conical, with a long spike about two to three cloacal body diameter. Bursa or bursal flap is absent.

**Female**

They are relaxed or slightly ventrally arcuate when killed by heat. Gonad is didelphic, amphidelphic. Each gonadal system is arranged from vulva/vagina as uterus, oviduct, and ovary. The anterior gonad is in 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 are mostly arranged in three to four rows in distal two-thirds of ovary and in double or single row in rest of the ovary, with distal tips of each ovary reaching oviduct of the opposite gonad branch. The anterior end of oviduct (= junction tissue between ovary and oviduct) consists of rounded cells. The anterior part of oviduct consists of rounded cells forming simple tube. The middle part of oviduct serves as spermatheca and consists of roundish and relatively large cells. Eggs in single to multiple-cell stage or even further stages are developed at the posterior part of oviduct (= uterus), which in young female is composed of squared or angular cells, long enough to contain one well-developed oocyte. *Receptaculum seminis* is not observed, i.e., the organ is not independent and is a part of oviduct/uterus. Vaginal glands are present but obscure. Vagina is perpendicular to the body surface, surrounded by sclerotized tissue. Vulva is slightly protuberant in lateral view and pore-like in ventral view. Rectum is about one anal body diameter long, intestine/rectum junction is surrounded by a well-developed sphincter muscle. Three anal glands (two subventral and one dorsal) are present but not obvious. Anus is in the form of dome-shaped slit, posterior anal lip slightly protuberant. Phasmid is about one to two anal body diameter long, posterior to anus. Tail is long, smoothly tapered to distal end variable from filiform to long and conical.

*Pristionchus paulseni* sp. n. (Figs 1, 2; Table 1).

**Measurements**

Summarized in Table 1.

**Description of species specific characters**

**Stenostomatous form**

The anterior end of cheilostomatal plates is slightly elongated to form a small rounded flap in most cases. However, in a few cases, the anterior end splits into two tips having two small flaps, i.e., regardless of the number of plates (= 6) resulting 6 to 12 flaps. Metastegostom bears a conspicuous and movable triangular or flint-shaped dorsal tooth with strongly sclerotized surface, giving an appearance of an inverted V-shape in light microscopy in lateral view; a pointed distal end, often curved to direct anteriorly; a pointed left subventral ridge with three minute adventitious denticles on a plate; and a pointed right subventral ridge, often with a distinct distal adventitious dentine.

MH dedicates the species name to Dr. MJ Paulsen of Lincoln, Nebraska, entomologist expert, friend, long-term expedition fellow and secret twin brother.
Figure 1: Adults of *Pristionchus paulseni* sp. n. A: female in right lateral view; B: male in right lateral view; C, D: anterior region of stenostomatous female (C) and male (D) in right lateral view showing the variation in body size and deirid position (arrow); E: body surface of female showing the relative position of deirid and lateral gland; F: postdeirid of female in left lateral view; G: phasmid opening of female in right lateral view; H: surface of male lip region in right lateral view; I: stomatal region of stenostomatal male in left lateral view; J, K: stomatal region of stenostomatal female in right lateral view showing the variation in cheilostomatal plates; L, M: stomatal region of eurystomatal female in left lateral view showing the variation in cheilostomatal plates; N: stomatal region of eurystomatal male in right lateral view showing partially split cheilostomatal plates; O: stomatal region of eurystomatal female in right lateral view.
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Eurystomatous form

Cheilostomatal plates are very thick. Anterior 1/3 to 1/2 sometimes split into two small tips. The anterior end of each plate is rounded and elongated to project from stomatal opening, forming a small flap; thus, the stoma has 6 to 12 flaps (=tips), regardless of the number of plates (=6). Each cheilostomatal plate is slightly inclined inwards, giving an appearance that the whole stoma is narrowing anteriorly. Gymnostom with very thick cuticle is present, forming a short, ring-like tube being thicker posteriorly and whole gymnostomatal ring widening anteriorly. The anterior end of gymnostom is internally overlapping the posterior end of cheilostomatal plates. Thus, the cheilo- and gymnostomatal regions form a barrel shape in lateral view. Metastegostom bears large claw-like dorsal tooth; large claw-like right subventral tooth; ridge of left subventral denticles or cusps, varying in number and size, i.e., three large cusps, the tip of these cusps is usually split into three or more small tips, sometimes forming serrated plate. The dorsal and right subventral teeth are movable. No movement is observed for left subventral denticles.

Figure 2: Adults of *Pristionchus paulseni* sp. n. A: male gonadal system in right lateral view; B; female anterior gonadal system in right lateral view; C, D: male tail region in right lateral view showing the variation in body size; E, F: male tail region in ventral view showing the variation in body size; G, H: spicule and gubernaculum in left lateral view showing the variation in body size; I: female vulval region in ventral view; J: female tail region in right lateral view; K: female anal region in ventral view.
### Table 1. Morpometrics *P. paulseni* sp. n. and *P. yamagatae* sp. n.

| Character                      | *P. paulseni* RS5918 | *P. paulseni* RS5964 | *P. yamagatae* RS5918 | *P. yamagatae* RS5964 |
|-------------------------------|----------------------|----------------------|-----------------------|-----------------------|
|                               | stenostomatous male  | stenostomatous female| stenostomatous male  | stenostomatous female |
| n                             | 10                   | 10                   | 10                    | 10                    |
| L                             | 1208 ± 95.4 (990–1315)| 1560 ± 230 (1222–1895)| 848 ± 42.2 (796–916) | 1162 ± 121.9 (937–1385) |
| L'                            | 1032 ± 92.5 (813–1138)| 1298 ± 209.6 (978–1560)| 681 ± 34.7 (616–724) | 908 ± 103.7 (717–1096) |
| a                             | 14 ± 1.3 (12–16)     | 14 ± 0.6 (13–15)     | 14 ± 1.4 (13–18)     | 14 ± 1.2 (12–17)      |
| b                             | 7.2 ± 0.8 (5.8–9.0)  | 8.7 ± 1.0 (7.3–10.1) | 5.8 ± 0.3 (5.3–6.2)  | 6.8 ± 0.6 (5.8–7.8)   |
| c                             | 6.9 ± 0.8 (5.6–8.3)  | 6.0 ± 0.6 (5.0–6.9)  | 5.1 ± 0.4 (4.4–5.9)  | 4.6 ± 0.4 (4.1–5.3)   |
| c'                            | 4.0 ± 0.6 (3.4–4.9)  | 6.0 ± 0.5 (5.3–6.8)  | 5.1 ± 0.6 (3.9–6)    | 7.3 ± 0.7 (6.1–8.2)   |
| T or V                         | 58 ± 2.9 (53–62)     | 47 ± 0.9 (45–48)     | 51 ± 3.3 (45–54)     | 45 ± 1.3 (44–48)      |
| Maximum body diam.            | 86 ± 4.7 (75–91)     | 114 ± 19.3 (84–138)  | 60 ± 6.4 (47–70)     | 84 ± 9.6 (68–98)      |
| Stoma length                  | 13.0 ± 0.5 (12.0–13.8)| 14.4 ± 0.4 (13.9–15.0)| 11.5 ± 0.7 (10–12.2) | 13.3 ± 0.5 (12.3–14) |
| Stoma diam.                   | 5.7 ± 0.5 (5.0–6.8)  | 6.8 ± 0.4 (6.2–7.5)  | 6 ± 0.5 (5.4–6.7)    | 7.4 ± 0.5 (6.7–8.3)   |
| Pharynx length (head to base of pharynx) | 156 ± 13.8 (122–168) | 169 ± 7.2 (158–178)  | 135 ± 5.4 (124–143)  | 156 ± 7.3 (146–170)   |
| Anterior pharynx (pro- + metacorpus) | 96 ± 8.3 (75–105)    | 106 ± 2.9 (102–110)  | 83 ± 3.9 (77–88)     | 98 ± 4.2 (93–106)     |
| Posterior pharynx (isthmus + basal bulb) | 61 ± 6.1 (47–68)    | 64 ± 4.8 (56–69)     | 52 ± 2.2 (47–55)     | 58 ± 3.9 (52–64)      |
| Ant/total pharynx %           | 61 ± 1.3 (59–63)     | 62 ± 1.6 (60–65)     | 63 ± 4.2 (59–74)     | 63 ± 1.2 (61–65)      |
| Median bulb diam.             | 28 ± 2.1 (25–31)     | 34 ± 2.8 (30–36)     | 22 ± 0.7 (21–23)     | 30 ± 3.8 (22–36)      |
| Terminal bulb diam.           | 26 ± 1.8 (23–28)     | 30 ± 3.9 (25–34)     | 21 ± 1.3 (18–22)     | 27 ± 3.7 (20–33)      |
| Testis length                 | 698 ± 69.9 (532–787) | –                    | 433 ± 37.9 (372–475) | –                     |
| Ant. end to vulva             | –                    | 730 ± 101.3 (592–860)| –                     | 527 ± 48.8 (446–610)  |
| Vulva to anus distance        | –                    | 570 ± 103.2 (424–710)| –                     | 380 ± 52.7 (281–470)  |
| Cloacal or anal body diam.    | 44 ± 3.4 (36–48)     | 44 ± 4.3 (37–50)     | 33 ± 3.6 (28–40)     | 35 ± 1.9 (32–38)      |
| Tail length                   | 176 ± 19.3 (155–220) | 262 ± 69.9 (235–335) | 167 ± 17.5 (140–192) | 255 ± 27.1 (212–289)  |
| Spicule length (curve)        | 51 ± 2.6 (47–54)     | –                    | 43 ± 1.2 (41–45)     | –                     |
| Spicule length (chord)        | 41 ± 2.5 (36–44)     | –                    | 36 ± 0.8 (34–37)     | –                     |
| Gubernaculum length           | 14 ± 1.3 (12–16)     | 14 ± 1.4 (13–18)     | –                     | –                     |
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Arrangement of genital papillae and phasmid

The paired papillae and the phasmid are arranged as \(<v1, v2, v3d, co, v4, ad, ph, (v5, v6, v7), pd>\), where \(v1\) is located at ca 1 CBD anterior to \(co\); \(v2\) and \(v3d\) are close to each other, but not overlapping, and \(v2\) is located at less than 1/2 CBD anterior to \(co\); \(v3d\) is at the midway between \(v2\) and \(co\); \(v4\) ca 1/2 CBD posterior to \(co\); \(v2\) and \(v3d\) are close to each other, but not overlapping, and \(v2\) located at less than 1/2 CBD anterior to \(co\); \(v3d\) at the midway between \(v2\) and \(co\); \(v4\) ca 1/2 CBD posterior to \(co\) vs. \(v1\) at 1.5 to 2 CBD anterior to \(co\), and adcloacal \(v2\) to \(v4\) are gathered within 1/2 CBD from \(co\), and finally the reproductive mode (Kanzaki et al., 2012a).

Type host (carrier) and locality

The culture from which the type specimens were obtained was originally isolated by M. Herrmann from an adult Lucanus dybowskii taiwanus collected at Pilu Sacred tree, Taroko National Park Taiwan in May 2017.

Type material, type strain, and nomenclatural registration

One slide with holotype male and two slides, each with paratypes one male and one female, 28533-28535, were deposited in the University of California Riverside Nematode Collection (UCRNC), CA. Two slides, each with paratypes one male and one female (SMNHType-8991 and 8992) were deposited in the Swedish Natural History Museum, Stockholm, Sweden. Two slides, each with paratypes one male and one female (SMNK-NEMA-T-0145 and 0146) were deposited in the Natural History Museum Karlsruhe, Germany. The strain is available as living culture and as frozen stocks under culture code RS5918 in the Department of Evolutionary Biology, Max Planck Institute (MPI) for Developmental Biology, Tübingen, Germany, and it can be provided to other researchers upon request. The new species binomial has been registered in the ZooBank database (zoobank.org) under the identifier [urn:lsid:zoobank.org:act:6E8BF33F-6F9E-4A6E-99C2-5576242EC26E].

Pristionchus yamagatae* sp. n. (Figs 3, 4; Table 1).

Measurements

Summarized in Table 1.

Description of species specific character

Stenostomatous form

The anterior ends of gymnostomatatal and pro-mesostegostomal rings are weakly serrated, but they are difficult to observe under light microscopy. Metastegostom
Figure 3: Adults of *Pristionchus yamagatae* sp. n. A: female in right lateral view; B: male in right lateral view; C, D: anterior region of eurystomatous (C) and stenostomatous (D) females in right and left lateral views, respectively; E: body surface of female showing the relative position of deirid and lateral gland; F: excretory pore opening in ventral view; G: phasmid opening of female in right lateral view; H: surface of male lip region in right lateral view; I: stomatal region of stenostomatal female in left lateral view; J: stomatal region of stenostomatal female in right lateral view; K: stomatal region of eurystomatal female in left lateral view showing partially split cheilostomatal plates; L: stomatal region of eurystomatal female in right lateral view.
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bears conspicuous and movable triangular or flint-shaped dorsal tooth with strongly sclerotized surface, giving an appearance of an inverted V-shape in light microscopy in lateral view; a pointed distal end often curved to direct anteriorly; a pointed left subventral ridge with three minute adventitious denticles on a plate; bluntly or sharply pointed right subventral ridge, often with a distinct distal adventitious denticle.

Figure 4: Adults of *Pristionchus yamagatae* sp. n. A: male gonadal system in right lateral view; B: female anterior gonadal system in right lateral view; C: female vulval region in ventral view; D: female tail region in left lateral view; E: female anal region in ventral view; F: surface striation of female anal region in ventral view; G: male tail region in left lateral view; H: male tail region in right lateral view showing the presence of extra v0 papilla (arrow); I: male tail region in ventral view; J: spicule and gubernaculum in right lateral view.
Eurystomatous form

Cheilostomatal plates are very thick. The anterior end of each plate is rounded and elongated to project from stomatal opening, forming a small flap. In the anterior part, only the tip to anterior half of each cheilostomatal plate sometimes splits into two small flaps, i.e., stomatal opening bearing 6 to 12 flaps (=tips), regardless of the number of plates (=6). Each cheilostomatal plate is slightly inclined inwards, giving an appearance that the whole stoma is narrowing anteriorly. Gymnostom with a very thick cuticle is present, forming a short, ring-like tube being thicker posteriorly, and whole gymnostomatral ring widening anteriorly. The anterior end of gymnostom is weakly serrated and it internally overlaps the posterior

Figure 5: Phylogenetic relationships of the Pristionchus genus. The schematic phylogeny shows the relationship between all species of the Pristionchus genus as inferred from transcriptome data (Rödelsperger et al., 2018), including those described in the two accompanying manuscripts (Yoshida et al., 2018; Kanzaki et al., 2018).
end of cheilostomatal plates. Thus, the cheilo and
and gymnostomatal regions form a barrel shape in later-
view. The anterior edge of pro-mesostegostom is
clearly serrated. Metastegostom bears a large claw-
like dorsal tooth; a large claw-like right subventral
tooth; ridge of left subventral denticles or cusps, var-
ying in number and size, i.e., three large cusps, the
tip of these cusps is usually split into three or more
small tips, sometimes forming serrated plate. The
dorsal and right subventral teeth are movable. No
movement is observed for left subventral denticles.

Arrangement of genital papillae
and phasmid

The paired papillae and the phasmid are arranged as
<v1, v2d, v3, co, v4, ad, ph, (v5, v6, v7), pd>, where v1
is located at ca 1 CBD or slightly more anterior to co;
v2d and v3 close to each other, but not overlapping,
and v2 is located at ca 1/3 CBD anterior to co; v3 at
the midway between v2d and co; v4 is less than 1/3
CBD posterior to co; thus, the distance between v2d
and v4 is less than 1 CBD; laterally located ad is at
the midway between co and the root of tail spike; ph
is subventrally located just anterior to or overlapping
with v5; ventral v5 to v7 is forming triplet just anterior
to the root of tail spike; and subdorsal pd is overlap-
ning or slightly posterior to v7. In addition to regular
nine pairs of papillae, a few individuals have an extra	pair of subventral papillae anterior to v1 (=v0).

Type host (carrier) and locality

The culture from which the type specimens were ob-
tained was originally isolated by N. Kanzaki from an
adult Holotrichia kyotoensis collected at a Mamuro-
gawa, Yamagata, Japan in July 2017.

Type material, type strain, and
nomenclatural registration

One slide with holotype male and two slides, each
with paratypes one male and one female, 28536–
28538, were deposited in the University of California
Riverside Nematode Collection (UCRNC), CA. Two
slides, each with paratypes one male and one female
(SMNHType-8993 and 8994), were deposited in the
Swedish Natural History Museum, Stockholm, Swed-
en. Two slides, each with paratypes one male and one
female (SMNKNEMA-T-0147 and 0148), were de-
posited in the Natural History Museum Karlsru-
he, Germany. The strain is available as living cultures
and as frozen stocks under culture code R5964 in
the Department of Evolutionary Biology, MPI for De-
velopmental Biology, Tübingen, Germany, and it can
be provided to other researchers upon request. The
new species binomial has been registered in the
ZooBank database (zoobank.org) under the identifier
[urn:lsid:zoobank.org:act:2A1FAEB0-0D51-4563-
A043-34B1515206A4].

Diagnosis and relationship

The diagnostic characters are described above. This
new species is molecularly characterized by the
barcoding sequence (NCBI accession number
MH114983). Also, the new species is typologically
distinctive from many other Pristionchus spp. with
its barrel-shaped stoma with weakly serrated ante-
rior end of gymnostomatal ring and clearly serrated
anterior end of pro-mesostegostomatal ring. These
specific characters suggest that P. yamagatae n.
sp. belongs to the P. triformis group including P. tri-
formis, P. hoplostomus and P. fukushimae. These
three species share the stomatal structure with the
new species (Ragsdale et al., 2013, 2015). Howev-
er, P. yamagatae n. sp. is readily distinguished from
P. triformis by its reproductive mode, gonochoristic
vs hermaphroditic, the cheilostomatal plates in the
stenostomatous and the eurystomatous forms, the
arrangement of male genital papillae, v2d and v3
being close to each other vs clearly separated, and
relative position of phasmid, located just anterior
to or overlapping with v5 vs being located midway
between ad and pd paired papillae, or more an-
teriorly, and clearly separated from v5 (Ragsdale
et al., 2013). The new species is distinguished from
P. hoplostomus by the cheilostomatal plates in the
stenostomatous form, i.e., the twelve-plated stenos-
tomatous form was not found, the arrangement of
genital papillae, the level of v4 being clearly apart
from cloacal opening vs close to cloacal opening, and
the relative position of phasmid, located just anterior
to or overlapping with v5 vs being located midway
between ad and pd paired papillae, or more ante-
iorly, and clearly separated from v5 (Ragsdale et al.,
2013). In addition to these typological characters, P. yamagatae sp. n. is
distinguished from its close relatives by its molecular
phylogenetic status and mating experiments.

Two new Species of Pristionchus (Nematoda: Diplogastridae) include the Gonochoristic Sister Species of P. fissidentatus
Molecular characterization and phylogenetic analysis

The phylogenetic positions of the two novel species were determined by phylogenetic analysis of transcriptomes from all cultivable *Pristionchus* species (Rödelsperger et al. 2018). The resulting phylogeny of the genus, as shown in Figure 5, places *P. yamagatae* sp. n. into the subclade consisting of *P. triformis*, *P. hoplostomus*, and *P. fukushimae*. More importantly, this phylogeny positions *P. paulseni* sp. n. as sister species to the hermaphroditic *P. fissidentatus*. Thus, except for *P. mayeri* and *P. boliviae*, all hermaphroditic species in the *Pristionchus* genus have at least one relatively close gonochoristic sister species.

Discussion

In this study, we have described two new *Pristionchus* species with the particular importance of *P. paulseni* sp. n. for two major reasons. First, *P. paulseni* sp. n. is most closely related to the hermaphroditic *P. fissidentatus*, a species that formerly had no known direct gonochoristic sister species. The comparison between *Pristionchus* hermaphrodites and their gonochoristic sister species allows important experimental inroads from mating-type-related traits such as longevity to comparative genomics (Weadick and Sommer, 2016; Rödelsperger et al., 2018). Therefore, the identification of *P. paulseni* sp. n. fills an important gap for the taxonomy and phylogeny of *Pristionchus* species and represents the fifth hermaphroditic–gonochoristic species pair in this genus.

Also, the species pair *P. paulseni* sp. n. and *P. fissidentatus* is the sister group to all described scarab beetle-associated *Pristionchus* species. Therefore, the characters of these species will be important for character polarization in the complete genus. For example, recent studies have started to investigate genome architecture across *Pristionchus* nematodes and basal gonochoristic species will be of critical importance (Rödelsperger et al., 2018). Also, the functional investigation of mouth-form plasticity in *P. pacificus* involves comparative studies including multiple *P. pacificus* strains and other *Pristionchus* species (for recent review see, Sommer et al., 2017). Ultimately, deep taxon sampling of basal species will result in a stronger support of character evolution. In the context of *Pristionchus* mouth-form plasticity, the fact of *P. paulseni* sp. n. and *P. fissidentatus* being dimorphic provides a strong support for plasticity being ancestral, whereas the single morphs known from *P. elegans* and *P. bucculentus* most likely represent examples of secondary losses. Thus, deep taxon sampling increases the robustness of *Pristionchus* phylogeny and character polarizations.

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References

Baskaran, P. et al. 2015. Ancient gene duplications have shaped developmental stage-specific expression in *Pristionchus pacificus*. BMC Evolutionary Biology 15:185. 12pp, online.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–7.

Ekseth, O. K., Kuiper, M. and Mironov, V. 2014. orthAgogue: an agile tool for the rapid prediction or orthology relations. Bioinformatics 30:734–6.

Haas B. J., Yassour M., Levin J. Z., Thompson D. A., Amit I., Adiconis X., Fan L., Raychowdhury R., Zeng Q., Chen Z., Mauceli E., Hacohen N., Gnirke A., Rhind N., di Palma F., Birren B. W., Nusbaum C., Lindblad-Toh K., Friedman N. and Regev A. 2011. Full length transcriptomics assembly from RNA-seq data without a reference genome. Nat. Biotechnol. 29:644–52.

Herrmann, M. et al. 2007. The nematode *Pristionchus pacificus* (Nematoda: Diplogastridae) is associated with the Oriental beetle *Exomala orientalis* (Coleoptera: Scarabaeidae) in Japan. Zoological Science 24:883–9.

Herrmann, M., Mayer, W. E. and Sommer, R. J. 2006. Nematodes of the genus *Pristionchus* are closely associated with scarab beetles and the Colorado potato beetle in Western Europe. Zoology 109:96–108.

Herrmann, M., Weiler, C., Rödelsperger, C., Kanzaki, N. and Sommer, R. J. 2016. Two new *Pristionchus* Species (Nematoda: Diplogastridae) from Taiwan are part of a species-cluster representing the closest known relatives of the Model Organism *P. pacificus*. Zoological Studies 55:48.

Hope, F. W. 1837. The Coleopterists manual, containing the lamellicorn insects of Linneus and Fabricius, London Henry G. Bohn.
Two new Species of Pristionchus (Nematoda: Diplogastridae) include the Gonochoristic Sister Species of P. fissidentatus

Kanzaki, N., Ragsdale, E., Herrmann, M. and Sommer, R. J. 2012a. Two new species of Pristionchus (Rhabditida: Diplogastridae) P. fissidentatus n.sp. from Nepal and La Réunion island and P. elegans n.sp. from Japan. Journal of Nematology 44:80–90.

Kanzaki, N., Ragsdale, E. J., Herrmann, M., Mayer, W. E. and Sommer, R. J. 2012b. Description of three Pristionchus species (Nematoda: Diplogastridae) from Japan that form a cryptic species complex with the model organism P. pacificus. Zoological Science 29:403–17.

Kanzaki, N., Ragsdale, E. J., Herrmann, M., Rösler, W. and Sommer, R. J. 2013. 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. Zoological Science 30:680–92.

Kanzaki, N., Ragsdale, E. J., Herrmann, M., Mayer, W. E., Tanaka, R. and Sommer, R. J. 2012c. Parapristionchus giblindavisi n. gen., n. sp. (Rhabditida: Diplogastridae) isolated from stag beetles (Coleoptera: Lucanidae) in Japan. Nematology 14:933–47.

Kanzaki, N., Herrmann, M., Yoshida, K., Weiler, C., Rödelsperger, C. and Sommer, R. J. 2013. 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. Zoological Science 30:680–92.

Kreis, H. A. 1932. Beiträge zur Kenntnis pflanzenparasitischer Nematoden. Zeitschrift für Parasitenkunde 5:184–94.

Miwa, Y. 1937. Descriptions of two new species of Lucanidae from Formosa. Transactions of the Natural History Society of Formosa 27:164–8.

Ragsdale, E., Kanzaki, N., Roeseler, W., Herrmann, M. and Sommer, R. J. 2013. Three new species of Pristionchus (Nematoda: Diplogastridae) show morphological divergence through evolutionary intermediates of a novel feeding polymorphism. Zoological Journal of the Linnean Society 168:671–98.

Ragsdale, E. J., Kanzaki, N. and Herrmann, M. 2015. Taxonomy and natural history: the genus Pristionchus. in Sommer, R. J. (Ed.), Pristionchus pacificus – a nematode model for comparative and evolutionary biology. BRILL, Leiden: 77–120.

Rödelsperger, C., Röseler, W., Prabh, N., Yoshida, K., Weiler, C., Herrmann, M. and Sommer, R. J. 2018. Phylotranscriptomics of Pristionchus nematodes reveals parallel gene loss in six hermaphroditic lineages. Current Biology, 28:3123–3127.

Sommer, R. J. 2015. Integrative evolutionary biology and mechanistic approaches in comparative biology. in Sommer, R. J. (Ed.), Pristionchus pacificus – a nematode model for comparative and evolutionary biology. BRILL, Leiden: 19–41.

Sommer, R. J., Carta, L. K., Kim, S. Y. and Sternberg, P. W. 1996. Morphological, genetic and molecular description of Pristionchus pacificus n. sp. (Nematoda: Neodiplogastridae). Fundamental and Applied Nematology 19:511–21.

Yoshida, K., Herrmann, M., Kanzaki, N., Weiler, C., Rödelsperger, C. and Sommer, R. J. 2018. Two new species of Pristionchus (Nematoda: Diplogastridae) from Taiwan and the definition of the pacificus species-complex sensu stricto. J. of Nematology 50. doi:10.21307/jofnem-2018-015.