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# ZOOTAXA



# Nine new Pristionchus (Nematoda: Diplogastridae) species from China

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# 名不正,则言不顺

"If names be not correct, language is not in accordance with the truth of things." (paraphrased as a chinese proverb stating "The beginning of wisdom is to call things by their proper name."). Confucius.

# Abstract

The model organism *Pristionchus pacificus* and the genus *Pristionchus*, Kreis, 1932 have been intensively studied in the last decade with contemporary work focusing on the development, evolution, ecology, behavior, neurobiology, and genomics of this group of organisms. In particular, mechanistic studies on the development and evolution of mouth-form plasticity, predation and associated self-recognition processes enabled unique insight into life history strategies and the evolution of novelty. These studies include a comparative research agenda making use of the 39 available species of *Pristionchus*, all of which can be studied in living cultures. Sampling efforts revealed that Asia represents a biodiversity hotspot for *Pristionchus* worms. However, previous samplings have a bias towards northern and island areas, largely for logistic reasons. Here, we report on two extensive sampling trips to the Yunnan and Shaanxi provinces in Mainland China. We report the isolation of nine new *Pristionchus* species by morphology, morphometrics, mating experiments and genome-wide sequence analysis.

Key words: Pristionchus pacificus, Diplogastridae, scarab beetles, phenotypic plasticity, evolution of hermaphroditism

# Introduction

The nematode *Pristionchus pacificus* Sommer, Carta, Kim & Sternberg, 1996 of the Diplogastridae family is a wellestablished model system in evolutionary developmental biology (Sommer 2009; Sommer 2015). A suite of analytical tools available for *P. pacificus* enable comparative and mechanistic studies building on a completely sequenced genome (Dieterich *et al.* 2008; Rödelsperger *et al.* 2017), comparative transcriptomics (Rödelsperger *et al.* 2018), DNA-mediated transformation (Schlager *et al.* 2009), and forward and reverse genetic tools, including the CRISPR/ Cas9 technology (Sommer *et al.* 1996; Tian *et al.* 2008; Witte *et al.* 2015). The isolation of hundreds of worldwide strains of *P. pacificus* (Morgan *et al.* 2012) and the support of a robust phylogenetic framework, consisting of more than 20 diplogastrid genera (Susoy *et al.* 2015, 2016), offer the potential to study the evolution of morphological, behavioral and life history traits within and among *Pristionchus* species.

In order to link processes of micro- and macroevolution, a precise knowledge of closely related species is a prerequirement, which is however, hard to achieve in many animal taxa. In the genus *Pristionchus*, the isolation of new species was fostered by the discovery that these soil nematodes are often associated with scarab and related beetles (Herrmann *et al.* 2006a, b, 2007). Therefore, directly targeting beetles for nematode sampling has led to a boom in

the number of Pristionchus strains and species recovered (Kanzaki et al. 2012a, b, 2013a, b, c, 2014; Ragsdale et al. 2013). Currently, 39 Pristionchus species have been described, all of which are cultured in the laboratory and are thus available for biological research (Ragsdale et al. 2015). This includes P. exspectatus Kanzaki, Ragsdale, Herrmann, Mayer & Sommer, 2012, a species close enough to be considered the true sister species of P. pacificus isolated in the South-Western part of Japan in 2010 (Kanzaki et al. 2012a). Both of these species are part of the pacificus-group sensu stricto, which currently consists of 10 species. In general, the genus Pristionchus is typologically and phylogenetically separated into six major radiations that have sometimes been refered to as "species-groups", "species-complex", or "clades" (Ragsdale et al. 2015; Herrmann et al., 2019). Specifically, there are i) the fig-associated clade that branches basal from all other beetle and soil-derived species, ii) another, beetle-associated basal radiation consisting currently of at least two small species groups, the *fissidentatus* and *elegans*-groups, respectively, iii) the triformis-group, iv) the lheritieri-group with species largely from Europe, v) the maupasi-group with species mainly from North-America, and vi) the pacificus-group. Within these groups, fig-associates have highly derived morphological and biological characters, e.g., the species have stomal polyphenism showing five different morphotypes, inhabit fresh syconia of figs in the subgenus Sycomorus and are vectored by fig wasps (Susoy et al., 2016). In contrast, all other species are morphologically very similar and only the use of sophisticated transcriptomic data allows proper phylogenetic groupings.

Reproductive biology within the genus *Pristionchus* is diverse. While the majority of *Pristionchus* species are gonochorists with equal numbers of males and females, hermaphroditism has arisen independently at least six times (Ragsdale *et al.* 2015; Weadick & Sommer 2016; Rödelsperger *et al.* 2018). Interestingly, only a few hermaphroditic species still form functional males, such as in the case of *P. pacificus*, representing a pre-requisite for genetic analysis. In contrast, other prominent hermaphrodites, such as the nearly cosmopolitan *P. maupasi* (Potts, 1910) Paramonov, 1952 and *P. entomophagus* (Steiner, 1929) Sudhaus & Fürst von Lieven, 2003, form only non-functional males, which prevents these species from being studied by forward genetic tools. Mating system transitions as seen between *P. pacificus* and *P. exspectatus* provide a powerful tool for comparative studies, as recently shown in the context of the evolution of life span and aging (Weadick & Sommer 2016). Therefore, the isolation and characterization of additional *Pristionchus* species is not only of taxonomic and systematic value, but offers unique opportunities for research in various areas of biology.

Asia is a hotspot of *Pristionchus* biodiversity and has recently been a rich source for novel *Pristionchus* species for two major reasons. First, most of the basal species of this genus and the outgroup *Parapristionchus* are from Asia (Kanzaki *et al.* 2012c, 2013a; Ragsdale *et al.* 2015). Second, all nine gonochoristic species of the *pacificus*-group *sensu stricto*, including *P. exspectatus* are from Asia, whereas the hermaphrodite *P. pacificus* is nearly cosmopolitan. Importantly, however, biogeographic studies of *Pristionchus* in Asia exhibit a sampling bias towards northern and island areas, and likely result in the current knowledge being incomplete (Kanzaki *et al.* 2012a, 2013a; Ragsdale *et al.* 2015). Mainland China was a *terra incognita* for diplogastrid nematodes, including the genus *Pristionchus*, until Li *et al.* (2015) described the first species. Subsequently, Yoshida *et al.* (2018), Kanzaki *et al.* (2018) and Herrmann *et al.* (2019) intensified the search for new *Pristionchus* species and described several species new to science from China. Realizing that China could be a treasure chest for new *Pristionchus* species, we conducted another two collecting trips with a focus on Yunnan and Shaanxi provinces. Here, we describe the results of these scarab beetle and substrate samplings and the resulting isolation of novel *Pristionchus* species from Mainland China in the years 2018 and 2019. Together, we describe nine new *Pristionchus* species by morphology, morphometrics, mating experiments and genome-wide sequence analysis.

# Material and methods

In July 2018, nine locations in Yunnan province were visited and 250 beetles from more than 16 genera, mostly from the Superfamily Scarabaeoidea were collected. Beetles were brought to the laboratory of the Max-Planck Institute (MPI) for Developmental Biology in Tübingen, Germany alive, where they were dissected and put on petri dishes with agar. These dishes were screened for two weeks for emerging nematodes. In July 2019, six locations in the Shaanxi province were sampled and yielded 585 beetles from around 20 genera. These beetles were treated the same way and emerging nematodes could be isolated from several individual beetles. Beetle identification was carried out by M.H. using morphological criteria.

**Isolation of isogenic nematode lines.** Nematodes were isolated from substrate or insects as single gravid females and transferred to Petri dishes containing NGM agar and *E. coli* (strain OP50) to generate isogenic lines.

**Morphological observation and type material preparation.** Light microscopic observations for drawings and morphometrics were conducted using live nematode material, which was handpicked from culture plates (Kanzaki 2013). 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). For scanning electron microscopy (SEM) observations, nematodes were prepared by fixation in 2.5% glutaraldehyde in M9 buffer and then post-fixation with 1% osmium tetroxide. After several rinses with water, samples were dehydrated through a graded ethanol series, followed by critical-point drying from carbon dioxide. Specimens were mounted on poly-lysine-coated cover-slips, sputter-coated with 20 nm gold/palladium, and then imaged with a Hitachi S-800 field emission scanning electron microscope operating at 20 kV.

To prepare type material, nematodes were isolated from cultures, rinsed in distilled water to remove bacteria, heat killed at 65°C, fixed in 5% formalin, and processed through a glycerol and ethanol series using Seinhorst's method (Hooper 1986).

Molecular characterisation and phylogenetic analysis. A species phylogeny of the complete Pristionchus genus was reconstructed and described previously (Rödelsperger et al. 2018). For the nine novel species described here, a similar molecular and transciptomic characterization was performed. In brief, 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 14 million paired reads ( $2 \times 150$  bp) 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).

**Intrageneric grouping of the genus** *Pristionchus*. The genus *Pristionchus* Kreis, 1932 is typologically redefined by Sudhaus and Fürst von Lieven (2003) and Kanzaki & Giblin-Davis (2015) as described below in more detail.

- 1) Buccal cavity often dimorphic (steno- and eurystomatous morphs)
- 2) Cheilostom with six per- and interradial flaps and separated into six per- and interradial plates, with the plates appearing stippled in lateral view (*i.e.*, right and left subventral, light and left lateral and right and left dorsal, sectors Cheilostomal plates of *Pristionchus* spp. are located between each sector, and the arrangement is regarded as per and interradial)
- 3) Stegostom of eurystomatous morph with dorsal claw-like tooth, right subventral tooth and left subventral serrated plate
- 4) Stegostom of stenostomatous morph with dorsal tooth, right subventral denticle and three left subventral denticles
- 5) Pharyngeal tubes of procorpus conspicuous
- 6) Female gonad amphidelphic
- 7) Gubernaculum with a paired thorn-like and an unpaired rounded anteriorly directed process

Recent dense taxon samplings followed by new species descriptions yielded several exceptional species. For example, three nominal and several unnamed fig-associated species have stomal polyphenism of up to five distinct morphs; two species have monomorphic stoma; and *triformis* group species have six to 12 cheilostomatal flaps and stenostomatous, eurystomatous and megastomatous morphs (Susoy *et al.* 2016; Kanzaki *et al.* 2018).

#### Results

Of the nine novel species to be described here, *P. nudus* **n. sp.** is the only one with several highly-derived, speciesspecific apomorphies, *e.g.* a relatively smooth body surface with a conspicuous lateral field and a less-developed median pharyngeal bulb. These typological characters do not fit the common characteristics of the genus *Pristionchus* under its current definition. In addition, the other two basal species, *P. paranudus* **n. sp.** and *P. chinensis* **n. sp.** also have some apomorphic characters. In the following, we first provide the description of the three basal species *P. nudus* **n. sp.**, *P. paranudus* **n. sp.** and *P. chinensis* **n. sp.** nudus **n. sp.**, *P. paranudus* **n. sp.** and *P. chinensis* **n. sp.** Next, we describe the common characters of the *triformis*group, followed by the description of four new species of this group. Finally, we describe the common characters of the *maupasi*-group and two new species belonging to this group.

## **Description of three basal species**

#### Pristionchus nudus n. sp.

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**Etymology.** The species name is derived from the absence of prominent striation, common in other *Pristionchus* species (*nudus* lat.: naked)

#### Measurements. See Table 1

Adult. Body cylindrical, stout, *i.e.*, body length-maximum body diam. ratio (a value) is ranging from 12-18, depending on culture condition; cuticle moderate in thickness, smooth with fine annulations, weak but conspicuous longitudinal striations, conspicuous lateral field with two bands, no clear annulation between bands. Head without apparent lips, six mound-like anteriorly directed expansions, one on each sector. Six labial sensilla one on each lip sector, long, bristle-like; two lateral sensilla clearly longer than other four. Cephalic papillae vestigial, probably degenerate and embedded in cephalic tissue. Amphidial apertures located on lateral sector, slightly dorsally shifted, at level of margin of cheilo- and gymnostom. Stomal dimorphism not observed; all individuals examined of stenostomatous form. Dorsal pharyngeal gland penetrating dorsal tooth to gland opening. Anterior pharynx (=pro and metacorpus) twice as long as posterior (=isthmus and basal bulb), muscular composed of tube-shaped procorpus and moderately developed metacorpus (median bulb) with roundish rectangular shape in lateral view; posterior pharynx glandular with nerve ring surrounding anterior end of isthmus. Pharyngo-intestinal junction (cardia) welldeveloped. Intestine simple tube, not forming pre-rectum, extended posteriorly from cardia to rectum; three (two subventral and one dorsal) rectal gland cells at distal end of intestine (margin between intestine and rectum). Secretory-excretory pore not conspicuous, ventrally located at level of isthmus to pharyngo-intestinal junction, excretory duct extending anteriad and reflexed back to position of pore; two large secretory-excretory cells around excretory duct. Deirid observed laterally on lateral field, located around posterior end of basal bulb to pharyngo-intestinal junction to a half body diameter posterior to junction, ca 0.5 body diam. posterior to secretory-excretory pore. Hemizonid not observed. Lateral glands (small pores connected to secretory cell) on lateral body surface, with positions inconsistent among individuals, numbering 5 to 8 for males and 9 to 13 for females. Postdeirid at anterior part of vas deferens in male and posterior end of posterior gonad in female, on the same striation with deirid (= lateral field) or the adjacent striation or the second dorsally neighboring striation to lateral field.

Stenostomatous form. Cheilostom consisting of six per- and interradial plates, each forming small flap at anterior end, posterior part of each plate broad, consisting of translucent and non-sclerotized tissue. Gymnostom short, cuticular ring-like anterior end overlapping cheilostom internally; two layers of metastegostomatal mounds consisting of weakly sclerotized tissue present on dorsal side of inner wall. Pro-meso stegostom forming a weakly cuticularized ring internally overlapping with gymnostom to connect gymnostom and metastegostom; broad and conspicuous in dorsal view. Metastegostom bearing dorsal mounds, small, conspicuous, triangular, movable tooth with clearly observed pharyngeal tube. Left subventral ridge with three minute, rounded adventitious denticles on a plate, and the most ventral denticle masked by remaining two in lateral view. Right subventral ridge with two rounded distal adventitious denticles. Telostegostom weakly sclerotized cup-like cavity connecting stoma and pharynx.

Male. Whole body ventrally arcuate, strongly ventrally curved at tail region when killed by heat. Testis single, ventrally located, anterior part reflexed to right or left side; spermatogonia arranged in three to five rows in reflexed

part, well-developed spermatocytes arranged as three to four rows in anterior two-thirds of main branch, mature amoeboid spermatids arranged in multiple rows in proximal part of gonad. *Vas deferens* not clearly separated from other parts of gonad. Posterior end of *vas deferens* and rectum fused to form a cloacal tube. Spicules paired, separate; spicules smoothly curved in ventral view, adjacent to each other for distal third of their length, each smoothly tapering to pointed distal end; spicule in lateral view smoothly ventrally arcuate, giving spicule about 100° curvature, oval manubrium at anterior end, lamina/calomus complex smoothly tapering to pointed distal end. Gubernaculum conspicuous, about one-third of spicule length, broad anteriorly such that dorsal wall is slightly recurved with dorsal and ventral walls separate at ca 30° angle at posterior end; dorsal side of gubernaculum possessing single,



**FIGURE 1.** *Pristionchus nudus* **n. sp.** A: Right lateral view of adult male. B: Right lateral view of adult female. C: Left lateral view of male head region showing labial sensilla, cephalic papillae and amphid. D: Ventral view of male head region showing labial sensilla, cephalic papillae, amphid and the anterior end of longitudinal striations. E-G: Stomal region of adult female in ventral (E), left lateral (F) and right lateral (G) view. Morphological variations of tooth and ridges are separately drawn in each subfigure as dorsal tooth (upper) and right and left subventral ridges (lower) (E), variation in left subventral ridge (left) and dorsal tooth (middle and right) (F) and variation of dorsal (left and middle) tooth and right subventral (right) ridge (G). H: Anterior part of adult female in left lateral view. I: Body surface structure showing lateral field and the relative position of deirid. J: Anterior female gonad in right lateral view. K: Vulval region in ventral view. L, M: Female tail in left lateral (L) and ventral (M) view. N, O: Male tail in ventral (N) and left lateral (O) view. P: Spicule and gubernaculum in left lateral view.



**FIGURE 2.** *Pristionchus nudus* **n. sp.** A: Left lateral view of stenostomatous form (only form observed) in four different focal planes. B: Right lateral view of stenostomatous form in two different focal planes. C: posterior pharynx region in three different focal planes in left lateral view where deirid (d), nerve ring (nr) secretory-excretory pore (ep) median bulb (mb) and basal bulb (bb) are indicated.

membranous, anteriorly directed process and lateral pair of more sclerotized, anteriorly and obliquely ventrally directed processes. In lateral view, anterior half of gubernaculum with two successive curves separated by anteriorly and obliquely ventrally directed process, with anterior terminal curvature highly concave and almost closed, with deep posterior curvature being one-third of gubernaculum length; posterior half forming tube-like process enveloping spicules. Cloacal opening (co) slit-like in ventral view; one small, ventral, single genital papilla (vs) on anterior cloacal lip. All nine paired genital papillae relatively long, bristle-like. Tail elongate conoid to sharply pointed tip, *i.e.*, tail does not form a distinct spike. Paired papillae and phasmid are arranged as <v1, v2d, v3, co, v4, ad, (v5, v6, v7), ph, pd>, where v1 located about 1 cloacal body diameter (CBD) anterior to co; v2d a little less than 0.5 CBD anterior to co; v3 adcloacal; v4 at less than 1/10 CBD posterior to co, *i.e.*, v3, co and v4 are very close to each other; ad about 1 CBD posterior to co; v5–v7 forming triplet, but clearly separated from each other, about 1 CBD posterior to ad, *i.e.*, about 2 CBD posterior to co; ph at about 1/4 CBD posterior to v7; and pd about 1 CBD posterior to v7. v1, v4 and ph subventral, v2d and ad lateral, v3, v5–7 ventral, pd subdorsal in male tail. Bursa or bursal flap absent.

Female. Body relaxed or weakly ventrally arcuate when killed by heat. Gonad didelphic, amphidelphic; each gonadal system arranged from vulva/vagina as uterus, oviduct, and ovary; anterior gonad right of intestine, with uterus and oviduct extending ventrally and anteriorly on right of intestine and with totally reflexed (=antidromous

reflexion) ovary extending dorsally on left of intestine; oocytes mostly arranged in three to four or more 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 end of oviduct (=junction tissue between ovary and oviduct) consists of rounded cells; anterior part of oviduct consists of rounded cells, forming a simple tube; middle part of oviduct serving as spermatheca, consists of roundish and relatively large cells. Eggs in single to multiple-cell stage or even further developed at posterior part of oviduct (=uterus), in young females being composed of squared or angular cells, long enough to contain one well-developed oocyte. *Receptaculum seminis* not observed, *i.e.*, the organ is not independent, and a part of oviduct/uterus works as the organ; vaginal glands present but obscure; vagina perpendicular to body surface, surrounded by sclerotized tissue; vulva slightly protuberant in lateral view, pore-like in ventral view; rectum about one anal body diameter (ABD) long, intestine/rectum junction surrounded by well-developed sphincter muscle. Anus in form of dome-shaped slit, posterior anal lip slightly protuberant; phasmid about 2 ABDs posterior to anus. Tail elongate, conoid, with sharply pointed tip.



**FIGURE 3.** *Pristionchus nudus* **n. sp.** male tail with the position of genital papillae labelled according to the terminology of Sudhaus and Fürst von Lieven (2003). A: lateral right view in six focal planes. B: ventral view in two focal planes.



**FIGURE 4.** Scanning electron photomicrograph of *Pristionchus nudus* **n. sp.** female anterior end with secretory-excretory pore (ep) being indicated.

**Diagnosis and relationships.** *Pristionchus nudus* **n. sp.** has several characteristic typological traits, which have not been found in other species of the genus. The new species is characterized by its long and bristle-like labial sensilla where two lateral sensilla are much longer than the other four, lack of male cephalic sensilla, monomorphic stoma morphology (stenostomatous form only), where thick posterior half of cheilostomal plate is composed by translucent tissue, two-layered dorsal gymno- and stegostomal mound, small and triangular dorsal tooth, round-ish rectangular-shaped metacorpus, position of nerve ring surrounding the anterior end of isthmus, long male tail without clear spike and the arrangement of male genital papillae, *i.e.*, <v1, v2d, v3, co, v4, ad, (v5, v6, v7), ph, pd>. The new species can be readily distinguished from all other *Pristionchus* species with the above species-specific characters, *i.e.*, none of the other species in the genus have these characters.

Phylogenetically, *P. nudus* **n. sp.** belongs to the basal species group of the genus, where two monomorphic species, *P. elegans* Kanzaki, Ragsdale, Herrmann, & Sommer, 2012 and *P. bucculentus* Kanzaki, Ragsdale, Herrmann, Röseler & Sommer, 2013 (*elegans* group), and two dimorphic species, *P. fissidentatus* Kanzaki, Ragsdale, Herrmann & Sommer, 2012 and *P. paulseni* Herrmann, Kanzaki, Weiler, Yoshida, Rödelsperger & Sommer, 2019 (*fissidentatus* group) have been described. *P. nudus* **n. sp.** shares the modification of the cheilostomatal plates with *P. elegans* and *P. bucculentus*, which have thin membrane-like cheilostomatal plates. Within these two species, *P. nudus* **n. sp.** shares the stoma pattern, monomorphic stenostomatous form with *P. elegans*. However, the new species is distinguished from *P. elegans* with its species-specific characters described above. Further, the new species is distinguished from all other species by mating experiments and also characterized by a *ca.* 1,600-bp fragment of the SSU rRNA gene (GenBank accession number MW017221), the sequence of which is distinct from that of all other *Pristionchus* species.

**Type host and locality.** Isolated from a longhorn beetle (Coleoptera: Cerambycidae) collected at Xishuangbanna Tropical Botanical Garden - Green stone forest, Yunnan province, PRC.

**Type material and type strain.** Type strain RS6026 frozen at the nematode collection of the MPI Tübingen and available as living culture upon request. Voucher specimens sent to the following museums: Holotype male, Para-type male and female: Museum für Naturkunde Karlsruhe, Germany; Paratype male and female: Swedish Natural History Museum, Stockholm, Sweden; Paratype male and female: University of California in Riverside Nematode Collection (UCRNC), Riverside, CA, USA.



**FIGURE 5.** Scanning electron photomicrograph of *Pristionchus nudus* **n. sp.** male *en face* with left and right amphids (la and ra) and left and right subventral (lsv and rsv), lateral (l and r) and left and right dorsal (ld and rd) labial sensilla being indicated.

# Pristionchus paranudus n. sp.

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Etymology. The species name is derived from its phylogenetic position as tentative sister to P. nudus.

# Measurements. See Table 1

Adult. Body cylindrical, moderate to stout, *i.e.*, body length-maximum body diam. ratio (a value) is ranging from 15–22, depending on culture condition; cuticle moderate in thickness, smooth with fine annulations, weak longitudinal striations, conspicuous lateral field with two bands. No clear annulation between bands. Head without apparent lips, six mound-like anteriorly directed expansions, one on each sector. Six labial sensilla one on each lip sector, short, papilliform. Four small papilliform cephalic papillae in males on right and left subventral and right and left dorsal sectors. Amphidial apertures located on the lateral sector, slightly dorsally shifted, at level of margin of cheilo- and gymnostom. Stomal dimorphism present, and details are described below. Dorsal pharyngeal gland clearly observed, penetrating dorsal tooth to gland opening. Anterior pharynx 1.5 times as long as posterior pharynx glandular with nerve ring surrounding the middle of isthmus. Pharyngo-intestinal junction (cardia) well-developed.



**FIGURE 6.** *Pristionchus paranudus* **n. sp.** A: Right lateral view of adult female. B: Right lateral view of adult male. C: Anterior part of adult female in left lateral view. D: Left lateral view of male head region showing labial sensilla, cephalic papillae and amphid. E, F: Stomal region of stenostomatous female in left (E) and right (F) lateral view. G, H: Stomal region of eurystomatous female in left (G) and right (H) lateral view. Morphological variations of tooth, ridges and serrated plate are separately drawn in each subfigure as left subventral ridge (left) and dorsal tooth (right) (E), dorsal tooth (left and middle) and right subventral ridge (right) (F), left subventral plate (left) and dorsal tooth (middle and right) (G), dorsal tooth (left) and right subventral plate (right) (H). I: Body surface structure showing lateral field and the relative position of deirid.



**FIGURE 7.** *Pristionchus paranudus* **n. sp.** A, B: Anterior female gonad of well-developed (A) and young (B) individual in right lateral view. C: Body surface structure at the postdeirid region in left lateral view showing lateral field and relative position of postdeirid. D, E: Female tail in right lateral (D) and ventral (E) view. F: Male gonad in left lateral view. G, H: Male tail in ventral (G) and left lateral (H) view. I: Spicule and gubernaculum in left lateral view.

Intestine simple tube, not forming pre-rectum, extended posteriorly from cardia to rectum; three (two subventral and one dorsal) rectal gland cells observed at distal end of intestine (margin between intestine and rectum). Secretory-excretory pore not conspicuous, ventrally located at level of isthmus to pharyngo-intestinal junction, excretory duct extending anteriad and reflexed back to position of pore; two large secretory-excretory cells around the excretory duct. Deirid observed laterally on lateral field, located at the level around the posterior end of basal bulb to pharyngo-intestinal junction to a half body diameter posterior to the junction, *ca* 0.5–1 body diam. posterior to secretory-excretory pore. Hemizonid not observed. Lateral glands (small pores connected to secretory cell) on lat-

eral body surface, with positions inconsistent among individuals, numbering 5 to 8 for males and 9 to 13 for females. Postdeirid at anterior part of *vas deferens* in male and the posterior end of posterior gonad in female, on the same striation with deirid (= lateral field) or on the adjacent striation or the second dorsally neighboring striation to lateral field.

Stenostomatous form. Cheilostom with six per- and interradial cuticular plates. Anterior end of each plate rounded and elongated to project from stomal opening and form a small flap. Gymnostom short, cuticular ring-like anterior end overlapping cheilostom internally with, metastegostomal mound consisting of weakly sclerotized tissue present on dorsal side of inner wall. Pro-meso stegostom forming a weakly cuticularized ring internally overlapping with gymnostoma to connect gymnostom and metastegostom, with dorsal side relatively thick and conspicuous, ventral side thin and inconspicuous. Metastegostom bearing dorsal mound, conspicuous, movable triangular or flint-shaped dorsal tooth with strongly sclerotized surface with an inverted V-shape in lateral view. Left subventral ridge with three minute, rounded adventitious denticles on a plate, most ventral denticle is masked by the remaining two in lateral view. Right subventral ridge with two rounded distal adventitious denticles. Telostegostom weakly sclerotized cup-like cavity connecting stoma and pharynx.



**FIGURE 8.** Stomal region of *Pristionchus paranudus* **n. sp.** A: Left lateral view of stenostomatous form in five different focal planes. B: Right lateral view of stenostomatous form in five different focal planes. C: Left lateral view of eurystomatous form in four different focal planes. D: Right lateral view of eurystomotous form in three different focal planes. E: Ventral view of stenostomatous form in five different focal planes. F: Ventral view of eurystomatous form in six different focal planes.

Eurystomatous form. Cheilostom divided into six per- and interradial plates. Anterior end of each plate rounded and elongated to project from stomal opening, forming a small flap. Tip of each cheilostomal plate sometimes split into two small flaps. Each cheilostomal plate inclined inwardly, *i.e.*, whole stoma appears to narrow anteriorly. Gymnostom with thick cuticle, forming short, ring-like tube with more heavily sclerotized wall in the posterior; anterior end of gymnostom internally overlapping posterior end of cheilostomatal plates. Pro-mesostegostom forming a weakly cuticularized ring internally overlapping with gymnostoma to connect gymnostom and metastegostom with dorsal side relatively thick and conspicuous, ventral side thin and inconspicuous; metastegostom bearing large claw-like or triangular dorsal tooth; two right subventral serrated plates; three left subventral serrated plates. Separation between serrated plates often inconspicuous. Telostegostom forming weakly sclerotized cup-like cavity connecting stoma and pharynx.



**FIGURE 9.** *Pristionchus paranudus* **n. sp.** A: posterior pharynx region in three different focal planes in left lateral view where deirid (arrowhead), nerve ring (nr) secretory-excretory pore (ep), median bulb (mb) and basal bulb (bb) are indicated. B: Left lateral view of male tail in three different focal planes, where genital papillae are labelled according to the terminology by Sudhaus & Fürst von Lieven (2003), and laterally located papillae are indicated with "d". C: Left lateral view of spicule and gubernaculum in six different focal planes.



**FIGURE 10.** Scanning electron photomicrograph of *Pristionchus paranudus* **n. sp.** stenostomatous male *en face* view where left amphid (la), left and right subventral (lsv and rsv), left and right lateral (l and r) and left and right dorsal (ld and rd) labial sensilla, and left and right subventral (lsvc and rsvc) and dorsal (ldc and rdc) cephalic sensilla are indicated.

Male. Whole body ventrally arcuate, strongly ventrally curved at tail region when killed by heat. Testis single, ventrally located, anterior part reflexed to right or left side; spermatogonia arranged in three to five rows in reflexed part, well-developed spermatocytes arranged as three to four rows in anterior two-thirds of main branch, mature amoeboid spermatids arranged in multiple rows in proximal part of gonad. Vas deferens not clearly separated from other parts of gonad. Posterior end of vas deferens and rectum fused to form a cloacal tube. Spicules paired, separate; spicules smoothly curved in ventral view, adjacent to each other for distal third of their length, each smoothly tapering to pointed distal end; spicule in lateral view smoothly ventrally arcuate, giving spicule about 100° curvature, oval manubrium at anterior end, lamina/calomus complex expanded slightly (ca 1/4 of blade length) posterior to manubrium, then smoothly tapering to pointed distal end. Gubernaculum conspicuous, about one-third of spicule length, broad anteriorly such that dorsal wall is slightly recurved with dorsal and ventral walls separate at 50 to 60° angle at posterior end; dorsal side of gubernaculum possessing single, membranous, anteriorly directed process and lateral pair of more sclerotized, anteriorly and obliquely ventrally directed processes. In lateral view, anterior half of gubernaculum with two successive curves separated by anteriorly and obliquely ventrally directed process, with anterior terminal curvature highly concave and almost closed, with deep posterior curvature being one-third of gubernaculum length; posterior half forming tube-like process enveloping spicules. Cloacal opening (co) slit-like in ventral view; one small, ventral, single genital papilla (vs) on anterior cloacal lip. All nine paired genital papillae relatively long, bristle-like. Tail conoid with long spike occupying more than 2/3 of tail length, possessing filiform terminus. Paired papillae and phasmid arranged as <v1, v2d, v3, co, v4, ad, ph, (v5, v6, v7), pd>, where v1 located about 1.5 CBD anterior to co; v2d midway between v1 and co; v3 adcloacal; v4 at less than 1/10 CBD posterior to co, *i.e.*, v3, co and v4 situated close to each other; ad less than 1 CBD posterior to co; ph at 1 CBD posterior to co; v5-v7 forming triplet, but clearly separated from each other, about 1 CBD posterior to ad, *i.e.*, less than 1/10 CBD posterior to ph; pd about 1/4 CBD posterior to v7. v1, v4 and ph subventral, v2d and ad lateral, v3, v5–7 ventral and pd subdorsal in male tail. Bursa or bursal flap absent.



**FIGURE 11.** Scanning electron photomicrograph of *Pristionchus paranudus* **n. sp.** male tail with everted spicula with the position of genital papillae according to the terminology of Sudhaus and Fürst von Lieven (2003).

Female. Body relaxed or weakly ventrally arcuate when killed by heat. Gonad didelphic, amphidelphic; each gonadal system arranged from vulva/vagina as uterus, oviduct, and ovary; anterior gonad right of intestine, with uterus and oviduct extending ventrally and anteriorly on right of intestine and with totally reflexed (=antidromous reflexion) ovary extending dorsally on left of intestine; oocytes mostly arranged in three to four or more 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 end of oviduct (=junction tissue between ovary and oviduct) consists of rounded cells; anterior part of oviduct consists of rounded cells, forming a simple tube; middle part of oviduct serving as spermatheca, consists of roundish and relatively large cells. Eggs in single to multiple-cell stage or even further developed at posterior part of oviduct (=uterus), in young females being composed of squared or angular cells, long enough to contain one well-developed oocyte. *Receptaculum seminis* not observed, *i.e.*, the organ is not independent, and a part of oviduct/uterus works as the organ; vaginal glands present but obscure; vagina perpendicular to body surface, surrounded by sclerotized tissue; vulva slightly protuberant in lateral view, pore-like in ventral view; rectum about one anal body diameter (ABD) long, intestine/rectum junction surrounded by well-developed sphincter muscle. Anus in form of dome-shaped slit, posterior anal lip slightly protuberant; phasmid about 2–2.5 ABDs posterior to anus. Tail long, smoothly elongate, conoid, with filiform terminus.

**Diagnosis and relationship.** *Pristionchus paranudus* **n. sp.** is characterized by its cuticle structure with weak longitudinal striation and distinctive lateral field, presence of partially split cheilostomatal plates and right and left subventral metastegostomatal serrated plates of eurystomatous form, long spike of male tail and the arrangement of male genital papillae, *i.e.*, <v1, v2d, v3, co, v4, ad, ph, (v5, v6, v7), pd>. The new species shares partially split

cheilostomatal plates with *P. paulseni* and some of the *triformis* group species (*P. hoplostomus* Ragsdale, Kanzaki, Röseler, Herrmann, Sommer, 2013; P. fukushimae Ragsdale, Kanzaki, Röseler, Herrmann, Sommer, 2013 and P. yamagatae Herrmann, Kanzaki, Weiler, Yoshida, Rödelsperger & Sommer, 2019), and its surface cuticle structure (moderate in thickness with weak striations and distinctive lateral field) and the structure and arrangement of male genital papillae (long bristle-like and ventrally located v3, co and subventral v4 are very close to each other) with P. nudus n. sp. However, the new species is distinguished from P. paulseni and triformis group by its left subventral stegostomal plate of the eurystomatous form, cuticle structure and the structure and the arrangement of genital papillae, i.e., P. paulseni and triformis group species have claw-like stegostomatous tooth on the left subventral sector of the eurystomatous form, thick cuticle with distinctive longitudinal striations and indistinctive lateral field, and v3 and v4 are clearly separated. The new species is also distinguished from *P. nudus* **n. sp.** by its median bulb, which is oval vs. somewhat rectangular in lateral view, the position of the nerve ring, middle vs. anterior end of isthmus, labial sensilla, papilliform vs. bristle-like, stomal morphology in stenostomatous form, with sclerotized chilostomatal plates, flint-shaped dorsal tooth with a metastegostomal mound on the dorsal side vs. thick and seemingly soft cheilostomatal plates, triangular dorsal tooth and two layers of dorsal metastegostomatal mounds, arrangement of v7), ph, pd>, and the male tail shape with vs. without spike. Further, the new species is distinguished from all other species by mating experiments and also characterized by a ca. 1,600-bp fragment of the SSU rRNA gene (GenBank accession number MW017222), the sequence of which is distinct from that of all other *Pristionchus* species.

**Type host and locality.** Isolated from rotting water hyacinth bulbs (*Eichhornia*) on terraces below Dayutang village, Yuanyang, Yunnan province, PRC.



**FIGURE 12.** Scanning electron photomicrograph of *Pristionchus paranudus* **n. sp.** posterior part of male tail and papillae with phasmid (ph) and paired papillae (v5–7, pd) indicated.

TABLE 1.				
	P. nuo	tus (RS6026)	P. paran	udus (RS5988)
character	stenostomatous male	stenostomatous female	stenostomatous male	stenostomatous female
n	5	5	5	5
L	$911 \pm 128.7 \ (704 - 1029)$	$1174 \pm 166.9 \ (911 - 1322)$	$761 \pm 79.6 \ (660 - 864)$	$990 \pm 89.7 \ (891 - 1106)$
Ľ	$748 \pm 117.7$ (572–872)	$956 \pm 101.1 \ (811 - 1079)$	$614 \pm 60.3 \ (553-696)$	733 ± 72.2 (674–825)
а	$16 \pm 1.1 \ (15-18)$	$14 \pm 1.2 \ (12 - 15)$	$18 \pm 3.0 \ (15-22)$	$18 \pm 1.5 \ (16-20)$
þ	$5.8 \pm 0.6 \ (4.8 - 6.4)$	$6.9 \pm 0.8 (5.7 - 7.9)$	$5.3 \pm 0.4 \ (4.7 - 5.7)$	$6.6 \pm 0.2 \ (6.3 - 6.9)$
c	$5.6 \pm 0.7 \ (4.6 - 6.5)$	$5.9 \pm 1.9 \ (4.4 - 9.1)$	$5.3 \pm 0.6 \ (4.6 - 6.2)$	$3.9 \pm 0.2 \ (3.6 - 4.1)$
ç	$3.7 \pm 0.4 \ (3.3 - 4.1)$	$4.6 \pm 1.1 \ (2.9 - 5.6)$	$4.8 \pm 1.1 \ (3.3 - 6.1)$	$7.3 \pm 0.7 \ (6.2 - 7.8)$
ant. stoma length (cheilo- + gymnostom)	$9.3 \pm 1.4 \; (7.4 - 10.4)$	$11.1 \pm 1.0 \ (10.2 - 12.7)$	$6.8 \pm 0.9 \ (5.9 - 8.0)$	$8.2 \pm 0.9 \ (6.9 - 9.1)$
total stoma length	$13.3 \pm 1.2 \ (12.2 - 15.0)$	$15.5 \pm 0.9 \ (14.5 - 17.0)$	$10.5 \pm 0.6 \ (9.8 - 11.2)$	$13.2 \pm 1.0 \ (11.6 - 14.4)$
stoma width	$7.3 \pm 0.8 \ (6.7 - 8.6)$	$7.9 \pm 0.5 \ (7.3 - 8.7)$	$5.0 \pm 0.9 \ (4.0 - 5.9)$	$6.7 \pm 0.9 \ (5.5 - 7.5)$
ant. pharynx length (pro- + metacorpus)	$93 \pm 5.1 \; (87 - 100)$	$106 \pm 5.2 \ (100 - 113)$	78 ± 3.1 (73−82)	$86 \pm 10.0 \ (78 - 103)$
post. pharynx length (isthmus + basal bulb)	$49 \pm 1.5 \; (47 - 50)$	$52 \pm 4.7 (48-59)$	$56 \pm 5.4 \ (49-62)$	$56 \pm 2.2 (53 - 59)$
total length pharynx	$142 \pm 5.4 \ (134 - 149)$	$158 \pm 5.2 \ (150 - 163)$	$133 \pm 8.0 \ (122 - 142)$	$142 \pm 11.6 \ (133 - 162)$
ant./total pharynx %	$66 \pm 1.3 \ (64-68)$	$67 \pm 2.7 \ (64 - 70)$	$58 \pm 1.8 (56-60)$	$60 \pm 2.2 \ (58-63)$
median bulb diameter	$21 \pm 1.8 \ (20 - 24)$	$28 \pm 3.1 \ (25 - 31)$	$21 \pm 2.4 \ (19-24)$	27 ± 3.0 (22–29)
terminal bulb diameter	$17 \pm 1.9 \; (16-20)$	$24 \pm 1.8 (22 - 27)$	$17 \pm 1.7 (15 - 19)$	$22 \pm 1.9 \ (18-23)$
neck length (incl. stoma)	$155 \pm 5.9 (146 - 162)$	$169 \pm 5.5 \ (161 - 174)$	$144 \pm 8.4 \ (132 - 152)$	$151 \pm 11.6 \ (141 - 169)$
excretion pore	$150 \pm 12.6 \ (131 - 162)$	$168 \pm 14.7 \ (154 - 186)$	$123 \pm 8.4 \ (115 - 132)$	$133 \pm 11.3 \ (119 - 145)$
nerve ring	$119 \pm 9.3 (105 - 131)$	$133 \pm 7.2 \ (122 - 141)$	$94 \pm 6.0 \ (85{-}101)$	$105 \pm 6.3 \ (98-112)$
testis length	$513 \pm 136.9 \ (325-656)$	ı	$376 \pm 18.2 \ (352 - 402)$	I
anterior gonad length	I	$273 \pm 40.8 (223 - 314)$	ı	$186 \pm 29.1 \ (161 - 234)$
posterior gonad length	I	$239 \pm 21.7 \ (211-260)$		$164 \pm 34.1 \ (136-207)$
ant. end to vulva distance	I	$566 \pm 63.3 \ (465-633)$		$436 \pm 38.5 \ (396 - 476)$
vulva to anus distance	I	$375 \pm 64.5 \ (281-445)$		$303 \pm 38.9 \ (268 - 357)$
T or V	$56 \pm 7.9 \ (46-64)$	$48 \pm 2.0 \ (46-51)$	$50 \pm 3.4 \ (46 - 53)$	$44 \pm 1.0 \ (43-45)$
max. body diameter	$56 \pm 5.3 (48-63)$	$85 \pm 12.6 \ (64-95)$	$43 \pm 3.6 \ (39-48)$	$57 \pm 6.1 \; (48-63)$
cloacal or anal body diameter	$44 \pm 7.9 \; (37 - 56)$	$46 \pm 9.0 (35-60)$	$31 \pm 2.9 \ (28 - 34)$	$35 \pm 4.4 \ (31 - 43)$
tail length	$162 \pm 23.8 \ (132 - 193)$	$218 \pm 72.7 \ (100-291)$	$147 \pm 24.8 \ (107 - 168)$	$256 \pm 23.4 \ (218-281)$
spicule length (curve)	$36 \pm 3.1 \; (31 - 40)$		$39 \pm 0.8 \ (38-40)$	
spicule length (chord)	$31 \pm 3.6 \ (25 - 34)$		$34 \pm 1.2 \ (33 - 35)$	
gubernaculum length	$17 \pm 2.7 (13-20)$		$15 \pm 1.1 \ (13-16)$	



FIGURE 13. Scanning electron photomicrograph of Pristionchus paranudus n. sp. surface structure with lateral line.

**Type material and type strain.** Type strain RS5988 frozen at the nematode collection of the MPI Tübingen and available as living culture upon request. Voucher specimen sent to the following museums: Holotype male, Para-type male and female: Museum für Naturkunde Karlsruhe, Germany; Paratype male and female: Swedish Natural History Museum, Stockholm, Sweden; Paratype male and female: University of California in Riverside Nematode Collection (UCRNC), Riverside, CA, USA.

#### Pristionchus chinensis n. sp.

urn:lsid:zoobank.org:act:2ECAEE4D-2089-48F7-83E0-86AA7F9591A5

Etymology. The species name is derived from China, the country of origin.

Measurements. See Table 2

Adult. Androdioecious species. Body cylindrical, stout, *i.e.*, body length-maximum body diam. ratio (a value) is usually ranging from 11–17, depending on culture condition; cuticle thick, with fine annulations, conspicuous longitudinal striations. Lateral field consisting of two lines, weakly separated from body striations by presence of deirid. Head without apparent lips, six mound-like anteriorly directed expansions, one on each sector. Six labial sensilla, one on each lip sector (on the mound); four small papilliform cephalic papillae in males on right and left subventral and right and left dorsal sectors; amphidial apertures located on lateral sector, slightly dorsally shifted, at level of margin of cheilo- and gymnostom. Stomal dimorphism present, and details are described below. Dorsal pharyngeal gland clearly observed, penetrating dorsal tooth to gland opening. Anterior pharynx 1.5 times as long as posterior pharynx, muscular, composed of tube-shaped procorpus, well-developed oval-shaped metacorpus (median bulb); posterior pharynx glandular with nerve ring surrounding middle of isthmus. Pharyngo-intestinal junction



**FIGURE 14.** *Pristionchus chinensis* **n. sp.** A: Right lateral view of adult female. B: Right lateral view of adult male. C: Anterior part of adult female in left lateral view including the body surface structure and relative position of deirid and postdeirid. D, E: Stomal region of stenostomatous female in left (D) and right (E) lateral view. F, G: Stomal region of eurystomatous female in left (F) and right (G) lateral view. Morphological variations of teeth, ridges and denticle are separately drawn in each subfigure as left subventral ridge (left) and dorsal tooth (right) (D), dorsal tooth (left and middle) and right subventral ridge (right) (E), left subventral denticles (left) and dorsal tooth (middle and right) (F), dorsal (left) and right subventral (right) teeth (G). H, I: Male tail in left lateral (H) and ventral (I) view. J: Spicule and gubernaculum in left lateral view. K–M: Female tail of large (K) and small (L) individuals in right lateral view, and ventral view (M).

(cardia) well-developed. Intestine simple tube, not forming pre-rectum, extended posteriorly from cardia to rectum; three (two subventral and one dorsal) rectal gland cells observed at distal end of intestine (margin between intestine and rectum). Secretory-excretory pore not conspicuous, ventrally located at level of isthmus to pharyngo-intestinal junction, excretory duct extending anteriad and reflexed back to position of pore; two large secretory-excretory cells around the excretory duct. Deirid observed laterally on lateral field, located at the level around the posterior

end of basal bulb to pharyngo-intestinal junction to a half body diameter posterior to the junction, ca 0.5 body diam. posterior to secretory-excretory pore. Hemizonid not observed. Lateral glands (small pores connected to secretory cell) on lateral body surface, with positions inconsistent among individuals, numbering 5 to 8 for males and 9 to 13 for females. Postdeirid at anterior part of *vas deferens* in male and the posterior end of posterior gonad in female, on the same striation with deirid (= lateral field) or on the adjacent striation or the second dorsally neighboring striation to lateral field.



**FIGURE 15.** Stomal region of *Pristionchus chinensis* **n. sp.** A: Left lateral view of stenostomatous form in four different focal planes. B: Right lateral view of stenostomatous form in three different focal planes. C: Left lateral view of eurystomatous form in four different focal planes. D: Right lateral view of eurystomatous form in two different focal planes.

Stenostomatous form. Cheilostom with six per- and interradial cuticular plates. Incision between plates not always distinguished. Anterior end of each plate rounded and elongated to project from stomal opening to form small flap. Gymnostom short, cuticular ring-like anterior end overlapping cheilostom internally; dorsal gymnostomal wall with mound-like expansion probably derived from metastegostom which gives an appearance of thickened dorsal gymnostomal wall compared to ventral side, and a metastegostomal mound consisting of weakly sclerotized tissue present on the dorsal side of the inner wall. Pro-meso stegostom forming a weakly cuticularized ring internally overlapping with gymnostoma to connect gymnostom and metastegostom. Metastegostom bearing dorsal mound and 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. Left subventral ridge with three minute, rounded adventitious denticles on a plate, and the most ventral denticle is masked by the other two in the lateral view. Right subventral ridge with three bluntly pointed distal adventitious denticles. Telostegostom forming weakly sclerotized cup-like cavity connecting stoma and pharynx.

Eurystomatous form. Cheilostom divided into six distinctive per- and interradial plates. Anterior end of each plate rounded and elongated to project from stomal opening, forming a small flap. Each cheilostomal plate inclined inwardly, giving an appearance that whole stoma is narrowing anteriorly. Gymnostom with thick cuticle, forming short, ring-like tube with more heavily sclerotized wall in the posterior. Pro-mesostegostom forming a weakly cu-ticularized ring connecting gymnostom and metastegostom. Metastegostom bearing large claw-like dorsal tooth; claw-like right subventral tooth; and three left subventral denticles, where the tip of each denticle sometimes splits into two or three small ridges. Telostegostom forming weakly sclerotized cup-like cavity connecting stoma and pharynx.

Male. Whole body ventrally arcuate, strongly ventrally curved at tail region when killed by heat. Testis single, ventrally located, anterior part reflexed to right or left side; spermatogonia arranged in three to five rows in reflexed part, well-developed spermatocytes arranged as three to four rows in anterior two-thirds of main branch, mature amoeboid spermatids arranged in multiple rows in proximal part of gonad. *Vas deferens* not clearly separated from other parts of gonad. Posterior end of *vas deferens* and rectum fused to form a cloacal tube. Spicules paired, separate; spicules smoothly curved in ventral view, adjacent to each other for distal third of their length, each smoothly

tapering to pointed distal end; spicule in lateral view smoothly ventrally arcuate, giving spicule about 100° curvature, oval manubrium at anterior end, lamina/calomus complex smoothly tapering to pointed distal end. Gubernaculum conspicuous, about one-third of spicule length, broad anteriorly such that dorsal wall is slightly recurved with dorsal and ventral walls separate at 50-60° angle at posterior end; dorsal side of gubernaculum possessing single, membranous, anteriorly directed process and lateral pair of more sclerotized, anteriorly and obliquely ventrally directed processes. In lateral view, anterior half of gubernaculum with two successive curves separated by anteriorly and obliquely ventrally directed process, with anterior terminal curvature highly concave and almost closed, with deep posterior curvature being one-third of gubernaculum length; posterior half forming tube-like process enveloping spicules. Cloacal opening (co) slit-like in ventral view; one small, ventral, single genital papilla (vs) on anterior cloacal lip. All nine paired genital papillae papilliform. Tail conoid with short spike. The paired papillae and the phasmid are arranged as <v1, v2d, v3, co, v4, ad, ph, (v5, v6, v7, pd)>, where v1 located about a little more than 1 CBD anterior to co; v2d midway between v1 and co; v3 1/4–1/5 CBD anterior to co; v4 at 1/4–1/5 CBD posterior to co; ad about 1 CBD posterior to co; ph at midway between ad and the root of tail spike; v5–v7 forming triplet, between ph and the root of tail spike; and pd at same level as v6 or v7. v1, v3, v4 and ph subventral, v2d and al lateral, v5–7 ventral, pd subdorsal in male tail. Bursa or bursal flap absent.



**FIGURE 16.** Male tail characters of *Pristionchus chinensis* **n. sp.** A: Left lateral view of male tail in seven different focal planes, where genital papillae are labelled according to the terminology by Sudhaus & Fürst von Lieven (2003), and laterally located papillae are indicated with "d", and rectal gland (rg) is labelled. B: Ventral view of the posterior part.

TABLE 2.		
		P. chinensis (RS6023)
character	stenostomatous male	stenostomatous hermaphrodite
n	5	5
L	$907 \pm 175.8 \ (703-1123)$	$1090 \pm 122.3$ (986–1289)
L'	$799 \pm 168.8 \ (604 - 1008)$	$929 \pm 120.6 \ (829 - 1129)$
а	$14 \pm 2.7 (11 - 17)$	$13 \pm 1.2 (11-14)$
b	$5.8 \pm 0.7 (5.0 - 6.4)$	$6.5 \pm 0.8 (5.9 - 7.9)$
C	$8.4 \pm 1.1$ (7.1–9.8)	$6.8 \pm 0.8 \ (6.2 - 8.0)$
c,	$2.4 \pm 0.4 \ (1.9-3.0)$	$4.0 \pm 0.5 \ (3.4 - 4.5)$
ant. stoma length (cheilo- + gymnostom)	$6.7 \pm 0.8 \ (5.8 - 7.4)$	$7.9 \pm 0.8 \ (6.9 - 8.9)$
total stoma length	$10.9 \pm 1.7 \ (8.3 - 12.3)$	$13.5 \pm 0.6 (12.6 - 14.2)$
stoma width	$7.7 \pm 0.8 \ (6.5 - 8.5)$	$8.4 \pm 0.4 \ (8.0-9.0)$
ant. pharynx length (pro- + metacorpus)	$78 \pm 4.6 (75-86)$	$93 \pm 2.4 \ (91 - 97)$
post. pharynx length (isthmus + basal bulb)	$67 \pm 9.1 (56-77)$	$67 \pm 3.0 \ (64 - 71)$
total length pharynx	$145 \pm 12.1 \ (133 - 163)$	$160 \pm 3.6 (155 - 163)$
ant./total pharynx %	$54 \pm 3.0 (50 - 58)$	$58 \pm 1.3$ (56–60)
median bulb diameter	$24 \pm 1.6 (23 - 27)$	$29 \pm 0.8 (29 - 30)$
terminal bulb diameter	$25 \pm 2.3 (22 - 27)$	$28 \pm 1.6 (26 - 30)$
neck length (incl. stoma)	$156 \pm 13.4 \ (141 - 175)$	$168 \pm 3.6 (164 - 171)$
excretion pore	$137 \pm 18.7 (113 - 156)$	$157 \pm 10.2 \ (148 - 173)$
nerve ring	$105 \pm 10.2 \ (93-119)$	$120 \pm 2.6 (118 - 124)$
testis length	$551 \pm 141.5 (404-765)$	
anterior gonad length	ı	$245 \pm 32.7 (212 - 291)$
posterior gonad length	1	$296 \pm 32.1 \ (251 - 337)$
ant. end to vulva distance	1	$533 \pm 36.1 (498-586)$
vulva to anus distance	1	$397 \pm 89.5 (327 - 549)$
T or V	$60 \pm 4.5 (57 - 68)$	$49 \pm 2.1 (45 - 51)$
max. body diameter	$68 \pm 9.7 (52 - 79)$	$88 \pm 18.9$ (74–121)
cloacal or anal body diameter	$46 \pm 5.9 (38-51)$	$41 \pm 4.7 (35 - 47)$
tail length	$107 \pm 7.1$ (99–115)	$161 \pm 8.8 (155 - 176)$
spicule length (curve)	$50 \pm 5.2$ (43–55)	
spicule length (chord)	$43 \pm 4.1$ (36–47)	
gubernaculum length	$16 \pm 1.9 (14 - 19)$	

Hermaphrodites. Body relaxed or weakly ventrally arcuate when killed by heat. Gonad didelphic, amphidelphic; each gonadal system arranged from vulva/vagina as uterus, oviduct, and ovary; anterior gonad right of intestine, with uterus and oviduct extending ventrally and anteriorly on right of intestine and with totally reflexed (=antidromous reflexion) ovary extending dorsally on left of intestine; oocytes mostly arranged in three to four or more 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 end of oviduct (=junction tissue between ovary and oviduct) consists of rounded cells; anterior part of oviduct consists of rounded cells, forming a simple tube; middle part of oviduct serving as spermatheca, consists of roundish and relatively large cells. Eggs in single to multiple-cell stage or even further developed at posterior part of oviduct (=uterus), in young females being composed of squared or angular cells, long enough to contain one well-developed oocyte. *Receptaculum seminis* not observed, *i.e.*, the organ is not independent, and a part of oviduct/uterus works as the organ; vaginal glands present but obscure; vagina perpendicular to body surface, surrounded by sclerotized tissue; vulva slightly protuberant in lateral view, pore-like in ventral view; rectum about one anal body diameter (ABD) long, intestine/rectum junction surrounded by well-developed sphincter muscle. Anus in form of dome-shaped slit, posterior anal lip slightly protuberant; phasmid about 1.5 ABDs posterior to anus. Tail conoid with or without elongated posterior half; and tail terminus sharply pointed.

**Diagnosis and relationships.** *Pristionchus chinensis* **n. sp.** is characterized by having the right subventral stegostomal ridge of stenostomatous form, with three bluntly pointed minute denticles, its relatively short tail in males and hermaphrodites, *i.e.* conoid with short (less than 1 CBD) spike in male and conoid with elongated posterior half in hermaphrodite, arrangement of male genital papillae, <v1, v2d, v3, co, v4, ad, ph, (v5, v6, v7, pd)>, and its characteristic reproductive mode, androdioecy.

*Pristionchus chinensis* **n. sp.** is typologically and phylogenetically close to *P. fissidentatus, i.e.*, these two species share the stomatal characters of stenostomatous form, relatively short male tail and the androdioecy, and further, both species belong to the basal clade of the genus. However, the new species can be distinguished from *P. fissidentatus* by its stomal structure of eurystomatous form, although these two species share the claw-like dorsal tooth and left subventral denticles with many minute cusps, the right subventral sector possessing a large claw-like tooth *vs.* ridge with three tips. Further, *P. chinensis* **n. sp.** can be distinguished from *P. fissidentatus* by its male tail characters of spontaneous males are sometimes inconsistent within species, the difference in the arrangement of genital papillae, <v1, v2d, v3, co, v4, ad, ph, (v5, v6, v7, pd) > vs. <math><v1, (co, v2), v3d, v4, ad, Ph, (v5, v6, v7), pd> and the length of tail spike less than *vs.* more than 1 CBD is consistent for these two species.

Further, the new species is distinguished from all other species by mating experiments and also characterized by a *ca.* 1,600-bp fragment of the SSU rRNA gene (GenBank accession number MW017217), the sequence of which is distinct from that of all other *Pristionchus* species.

**Type host and locality.** From *Mimela* sp. (Coleoptera: Rutelidae) collected at "Skytree" near Bubeng Fieldstation CAS, Yunnan province, PRC.

**Type material and type strain.** Type strain RS6023 frozen at the nematode collection of the MPI Tübingen and available as living culture upon request. Voucher specimens sent to the following museums: Holotype male, Paratype hermaphrodite: Museum für Naturkunde Karlsruhe, Germany; Paratype hermaphrodite: Swedish Natural History Museum, Stockholm, Sweden; Paratype hermaphrodite: University of California in Riverside Nematode Collection (UCRNC), Riverside, CA, USA.

#### Species description of four Pristionchus triformis-group species

Common characters. The basal species groups described above are highly variable in the basic typological characters, *e.g.*, the presence/absence of stomal dimorphism, cuticle structure, pharyngeal structure and position of the nerve ring. In comparison, species in the *triformis*-group are uniform, and they share common characters as described below, based on re-observation of four previously described species (*P. triformis* Ragsdale, Kanzaki, Röseler, Herrmann, Sommer, 2013, *P. hoplostomus*, *P. fukusimae* and *P. yamagatae*) and three new species. Several diagnostic characters are summarized in Table 3.

Adult. Body cylindrical, stout, *i.e.*, body length-maximum body diam. ratio (a value) is usually ranging from 10–18, depending on culture condition; cuticle with fine annulation, clear longitudinal striations and indistinct lateral field consisting of two lines, weakly separated from the body striations by presence of deirid, and absence of clear annulations. Head without apparent lips, six mound-like anteriorly directed expansions, one on each sector.



**FIGURE 17.** Stomal morphology of six *triformis*-group species. The left and right lateral views of three morphotypes are shown. Instead of the six-plated eurystomatous form, the 12-plated (megastomatous) form is drawn for *P. triformis*.

Species	Reproductive	Stenostom	atous form	Eurystom	atous form	Genital papillae	Other characteristic feature(s)
	mode	Right subventral	Left subventral nlate	Right subventral	Left subventral		
triformis	H	Single peak, sometimes very	Three or four bumps	Claw-like, single peak	Three plates with various number of	v1-v2d ≤ v2d-v4; v2d and v3 clearly separated; v4-ad <	12-plated (megastomatous) form present
hoplostomus	U	well-developed 1-3 peak(s)	Three bumps	Claw-like, 1-2 peak(s)	cusps Three plates with various number of cusps	ad-pd v1-v2d $\geq$ v2d-v4; v2d and v3 close to each other; v4-ad = ad-pd	Dorsal tooth of stenostoma- tous form sometimes dorsally curved and anteriorly directed.
fukushimae	Ċ	0-3 peak(s)	Three bumps	Claw-like, single peak	Three plates with various number of	$v1-v2d \ge v2d-v4$ ; $v2d$ and $v3$ close to each other; $v4-ad =$	Twelve-plated stenostomatous form present, but rare.
yamagatae	IJ	Single peak	Three bumps	Claw-like, 1-2 peak(s)	cusps Three plates with various number of	ad-pd v1-v2d $\geq$ v2d-v4; v2d and v3 close to each other; v4-ad =	Dorsal tooth of stenostomatous form somewhat blunt
magnoliae n. sp.	U	Two peaks	Three bumps	Claw-like, 1-2 peak(s)	cusps Three plates with various number of	ad-pd v1-v2d > v2d-v4; v2d and v3 close to each other; v4-ad =	Dorsal tooth of stenostomatous form somewhat blunt.
<i>musae</i> n. sp.	IJ	Two peaks	Three bumps	Claw-like, 1-2 peak(s)	Three plates with various number of	vu-pu v1-v2d > v2d-v4; v2d and v3 close to each other; v4-ad =	Dorsal tooth of stenostoma- tous form sometimes dorsally
auriculatae n. sp.	IJ	One or two blunt peak(s)	Three bumps	Claw-like, 1-2 peak(s)	Three plates with various number of cusps	v1-v2d = v2d-v4; v2d and v3 close to each other; v4-ad = ad-bd	Dorsal tooth of stenostoma- tous form sometimes dorsally curved and anteriorly directed.
passalidorum n. sp.	IJ	Single peak	Three or more denticles (pointed)	Claw-like, single peak	Three plates with various number of cusps	v1-v2d > v2d-v4; v2d and v3 close to each other; v4-ad = ad-pd	Dorsal tooth of stenostoma- tous form sometimes dorsally curved and anteriorly directed.

Six labial sensilla one on each lip sector, papilliform, and four male cephalic papillae papilliform, on each of right and left subventral and right and left dorsal lip sectors, smaller than labial sensilla. Stomal dimorphism (or polymorphism) present, and its details are described below. Anterior pharynx 1.5 times as long as posterior pharynx, muscular composed of tube-shaped procorpus and well-developed oval-shaped metacorpus (median bulb); posterior pharynx glandular with nerve ring at mid-isthmus length. Pharyngo-intestinal junction (cardia) clearly observed, well-developed. Intestine simple tube, not forming pre-rectum, extended posteriorly from cardia to rectum; three (two subventral and one dorsal) rectal gland cells observed at distal end of intestine (margin between intestine and rectum). Secretory-excretory pore not conspicuous, ventrally located at level of isthmus to pharyngo-intestinal junction, excretory duct extending anteriad and reflexed back to position of pore; two large secretory-excretory cells around the excretory duct. Deirid observed laterally on lateral field, located at the level around the posterior end of basal bulb to pharyngo-intestinal junction to a half body diameter posterior to the junction, ca 0.5–1 body diam. posterior to secretory-excretory pore. Hemizonid not observed. Lateral glands (small pores connected to secretory cell) on lateral body surface, with positions inconsistent among individuals, numbering 5 to 8 for males and 9 to 13 for females. Postdeirid at anterior part of vas deferens in male and the posterior end of posterior gonad in female, on the same striation with deirid (= lateral field) or on the adjacent striation or the second dorsally neighboring striation to lateral field.

Stenostomatous form. Cheilostom consisting of six per- and interradial plates. Each plate is sometimes separated into two plates, forming a rare 12-plated morph. Incision between plates not always easily distinguished. Anterior end of each plate rounded and elongated to project from stomal opening forming small flap. Gymnostom short, cuticular ring-like anterior end overlapping cheilostom internally. Stegostom separated into three subsections: pro-meso, meta, and telostegostom. Pro-mesostegostom forming a weakly cuticularized ring internally overlapping with gymnostoma to connect gymnostom and metastegostom. Metastegostom bearing 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; pointed left subventral ridge with three minute adventitious denticles on a plate, most ventral denticle often masked by remaining two in lateral view; pointed right subventral ridge, often with distinct distal adventitious denticle(s). Shape of dorsal tooth, right and left subventral ridges are variable within and among species. Telostegostom weakly sclerotized cup-like cavity connecting stoma and pharynx.

Eurystomatous form. Cheilostom divided into six well-distinguished per- and interradial plates. Anterior end of each plate rounded and elongated to protrude from stomal opening and form a small flap. Plates often partially or completely separated to form large plates with split tip or two narrow plates. 6–12 cheilostomatal flaps derived from 6–12 plates. 12-plated eurystomatous form of *P. triformis* is regarded as 'megastomatous form'. Gymnostom with thick cuticle, forming short, ring-like tube being thicker posteriorly; finely serrated anterior end of gymnostom internally overlapping posterior end of cheilostomatal plates. Structural variation within each species described below. Stegostom separated into three subsections: pro-meso, meta, and telostegostom bearing large claw-like dorsal tooth, and large, claw-like or pointed right subventral tooth. Left subventral sector of metastegostom bearing three triangular ridges; tip of each ridge sometimes split into two or more fine tips, shape varies within and among species. Dorsal tooth and right subventral tooth movable. Movement not observed in left subventral denticles. Telostegostom weakly sclerotized cup-like cavity connecting stoma and pharynx.

Variation in gymnostom and pro-mesostegostom of eurystomatous form.

Pro-mesostegostom of *triformis*-group well-developed compared to other species in genus, two different types of stomal structure in eury- and megastomatous forms. Relatively well sclerotized pro-mesostegostom internally overlap with posterior end of gymnostom. In one type, referred to as 'type 1', gymnostom relatively thick and short, pro-mesostegostom relatively short, bearing coarse and short serrates at anterior end (Fig. 21D). 'Type 2' with thin and long gymnostom, pro-mesostegostom loses anterior serrates, but often bears sparse or 2–3 rows of fine serrates on inner wall of elongated pro-mesostegostom. These two types are not always easily distinguishable by light microscopy. Further, intermediate types, *e.g.*, lacking both anterior and posterior serrates, occur. Both types are treated as variation within the eury/megastomatous forms.

Male. Whole body ventrally arcuate, strongly ventrally curved at tail region when killed by heat. Testis single, ventrally located, anterior part reflexed to right or left side; spermatogonia arranged in three to five rows in reflexed part, well-developed spermatocytes arranged as three to four rows in anterior two-thirds of main branch, mature amoeboid spermatids arranged in multiple rows in proximal part of gonad. *Vas deferens* not clearly separated from

other parts of gonad. Posterior end of *vas deferens* and rectum fused to form a cloacal tube. Spicules paired, separate; spicules smoothly curved in ventral view, adjacent to each other for distal third of their length, each smoothly tapering to pointed distal end; spicule in lateral view smoothly ventrally arcuate, giving spicule about 100° curvature, oval manubrium at anterior end, lamina/calomus complex smoothly tapering to pointed distal end. Gubernaculum conspicuous, about one-third of spicule length, broad anteriorly such that dorsal wall is slightly recurved with dorsal and ventral walls separate at 50–60° angle at posterior end; dorsal side of gubernaculum possessing single, membranous, anteriorly directed process and lateral pair of more sclerotized, anteriorly and obliquely ventrally directed processes. In lateral view, anterior half of gubernaculum with two successive curves separated by anteriorly and obliquely ventrally directed process, with anterior terminal curvature highly concave and almost closed, with deep posterior curvature being one-third of gubernaculum length; posterior half forming tube-like process enveloping spicules. Cloacal opening (co) slit-like in ventral view; one small, ventral, single genital papilla (vs) on anterior cloacal lip. All nine paired genital papillae papilliform. Tail conoid with a long spike. Arrangement of paired papillae and phasmid is variable among species, but size and structure of papillae are consistent with what has been described above. Bursa or bursal flap absent.

Female/hermaphrodite. Body relaxed or weakly ventrally arcuate when killed by heat. Gonad didelphic, amphidelphic; each gonadal system arranged from vulva/vagina as uterus, oviduct, and ovary; anterior gonad right of intestine, with uterus and oviduct extending ventrally and anteriorly on right of intestine and with totally reflexed (=antidromous reflexion) ovary extending dorsally on left of intestine; oocytes mostly arranged in three to four or more 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 end of oviduct (=junction tissue between ovary and oviduct) consists of rounded cells; anterior part of oviduct consists of rounded cells, forming a simple tube; middle part of oviduct serving as spermatheca, consists of roundish and relatively large cells. Eggs in single to multiple-cell stage or even further developed at posterior part of oviduct (=uterus), in young females being composed of squared or angular cells, long enough to contain one well-developed oocyte. *Receptaculum seminis* not observed, *i.e.*, the organ is not independent, and a part of oviduct/uterus works as the organ; vaginal glands present but obscure; vagina perpendicular to body surface, surrounded by sclerotized tissue; vulva slightly protuberant in lateral view, pore-like in ventral view; rectum about one anal body diameter (ABD) long, intestine/rectum junction surrounded by well-developed sphincter muscle. Anus in form of dome-shaped slit, posterior anal lip slightly protuberant. Tail elongate conoid with filiform terminus. The position of phasmid described for each species.

#### Pristionchus magnoliae n. sp.

urn:lsid:zoobank.org:act:ADA242D3-23FE-4ED0-AE51-6A537385AF79

**Etymology.** The species name is derived from the associated substrate from which the species was recovered. The strain was isolated from rotting *Magnolia grandiflora* fruits with some soil material.

#### Measurements. See Table 4

Adult. General characters are as described above for the triformis-group.

Stenostomatous form. Cheilostom, gymnostom, pro-mesostegostom and telostegostom as described above. 12plated form not found. Arrangement of tooth and denticles in metastegostom as described above; dorsal movable tooth somewhat narrow, *i.e.*, the angle of anteriorly detected tip is steeper, triangular and slightly anteriorly directed in male, flint-shaped with somewhat blunt tip in female, both have strongly sclerotized surface giving an appearance of an inverted V-shape in lateral view; left subventral ridge with three minute, blunt adventitious denticles on plate; right subventral ridge with three distal rounded adventitious denticles, plate slightly narrower in male than female, thus males seems to have two pointed denticles.

Eurystomatous form. Cheilostom as described above, anterior half of each cheilostomal plate often split into two tips to form 12-flapped form. Six-flapped form (without split cheilostomal plate) rare. Gymnostom in both types 1 and 2 short and thick, forming cuticular ring. Pro-mesostegostom well-developed, internally overlapping with the posterior end of gymnostom; type 1 form short, somewhat flattened and bearing weak serrates at anterior end; type 2 with 2–3 rows of small spines on inner surface. Arrangement of tooth and denticles in metastegostom as described above; dorsal movable tooth claw-like as typical of the genus; left subventral ridge with three large plates, each often has split tips and extra denticles on middle forming spiny plate; right subventral movable tooth claw-like and often bears an extra peak on the ventral side. Telostegostom as described above.

Male. Paired papillae and phasmid are arranged as <v1, v2d, v3, co, v4, ad, ph, (v5, v6, v7), pd>, where v1 located about a little more than 1 CBD anterior to co; v2d less than 1/4 CBD anterior to co; v3 adcloacal; v4 at 1/4–1/5 CBD posterior to co, *i.e.*, v2d, v3, co and v4 are close to each other; ad about 1 CBD posterior to co; ph at midway between ad and root of tail spike; v5–v7 forming triplet, between ph and the root of tail spike; and pd around level of v7. v1, v3, v4 and ph subventral, v2d and ad lateral, v5–7 ventral, pd subdorsal in male tail. General shape of spicule and gubernaculum as described above.



**FIGURE 18.** *Pristionchus magnoliae* **n. sp.** A: Right lateral view of adult female. B: Right lateral view of adult male. C: Anterior part of adult female in right lateral view. D: Body surface structure showing lateral gland, deirid, postdeirid and relative position of deirid and postdeirid. E: Left lateral view of male head region showing labial sensilla, cephalic papillae and amphid. F, G: Stomal region of stenostomatous male in left (F) and right (G) lateral view. H, I: Stomal region of stenostomatous female in left (J) and right (I) lateral view. J, K: Stomal region of eurystomatous female with pro-mesostegostomatal spines (type 2) in left (J) and right (N) lateral view. L–N: Stomal region of eurystomatous female with pro-mesostegostomatal serrates (type 1) in left (L, M) and right (N) lateral view where six-flapped form (L) is also shown. Morphological variations of teeth and ridges are separately drawn in each subfigure as left subventral ridge (left) and dorsal tooth (right) (G), dorsal tooth (left) and right subventral ridge (right) (H), left subventral ridge (left) and dorsal tooth (right) (I), left subventral ridge (left) and dorsal tooth (right) (L), left subventral ridge (left) and right subventral ridge (left) and right subventral ridge (left) and right) (M), and dorsal (left) and right subventral ridge (left) and right) (M), and dorsal (left) and right subventral ridge (left) and right) (M), and dorsal (left) and right subventral ridge (left) and right) (M), and dorsal (left) and right subventral ridge (left) and right) (M), and dorsal (left) and right subventral ridge (left) teeth (N).



**FIGURE 19.** *Pristionchus magnoliae* **n. sp.** A: Anterior female gonad of well-developed individual in right lateral view. B: Vulval region in ventral view. C, D: Female tail in left lateral (C) and ventral (D) view. E: Male gonad in right lateral view. F, G: Male tail in ventral (F) and left lateral (G) view. H: Spicule and gubernaculum in left lateral view.



**FIGURE 20.** Stomal region of *Pristionchus magnoliae* **n. sp.** A: Left lateral view of stenostomatous form in five different focal planes. B: Right lateral view of stenostomatous form in two different focal planes. C: Left lateral view of type 2 eurystomatous form in three different focal planes. D: Right lateral view of type 1 eurystomatous form in two different focal planes. E: Left lateral view of six-plated eurystomatous form in two different focal planes.

Female. Gonadal characters of female as described above. Tail elongate conoid with slightly filiform terminus, *i.e.*, the posterior half of tail more elongated compared with anterior part. Phasmid ventro-laterally located at about 1.0–1.5 ABD posterior to anal opening.

**Diagnosis and relationships.** Pristionchus magnoliae **n. sp.** is characterized by the somewhat blunt and flintshaped dorsal tooth and the right and left subventral ridges with three blunt denticles of stenostomatous form, right subventral stegostomal tooth which often has blunt peak on ventral side in eurystomatous form, well-serrated promesostegostomal wall in type 2 eurystomatous form, arrangement of male genital papillae, <v1, v2d, v3, co, v4, ad, ph, (v5, v6, v7), pd> where v2d, v3, co and v4 are close to each other, and a long spike occupying more than 2/3of tail length. *Pristionchus magnoliae* **n. sp.** is typologically close to *P. yamagatae* and *P. hoplostomus*. The new species and the other two species share the arrangement of genital papillae, <v1, v2d, v3, co, v4, ad, ph, (v5, v6, v7), pd> where v2d, v3, co and v4 are close to each other, and right subventral tooth in eurystomatous form, often having an extra peak on its ventral side. In addition, somewhat blunt and flint-shaped dorsal tooth in the stenostomatous form is common in the new species and P. yamagatae. However, the new species is distinguished from P. hoplostomus by the absence vs. presence of 12-plated stenostomatous form and dorsal tooth of stenostomatous form, blunt vs. pointed. The typological characters of *P. magnoliae* **n. sp.** are almost identical with those of *P. yamagatae*, distinguished only by the relative position of ph and v5, close but clearly separated vs. very close and sometimes overlapping. Further, the new species is distinguished from all other species by mating experiments and also characterized by a ca. 1,600-bp fragment of the SSU rRNA gene (GenBank accession number MW017219), the sequence of which is distinct from that of all other Pristionchus species.

Type host and locality. Rotting Magnolia grandiflora fruits in Changfeng Park, Shanghai, PRC.

**Type material and type strain.** Type strain RS5999, other strain RS 6000 from the same location, frozen at the nematode collection of the MPI Tübingen and available as living culture upon request. Voucher specimens sent to the following museums: Holotype male, Paratype male and female: Museum für Naturkunde Karlsruhe, Germany; Paratype male and female: Swedish Natural History Museum, Stockholm, Sweden; Paratype male and female: University of California in Riverside Nematode Collection (UCRNC), Riverside, CA, USA.



**FIGURE 21.** Male tail characters of *Pristionchus magnoliae* **n. sp.** Left lateral view of whole tail in five different focal planes and of spicule and gubernaculum in two different focal planes. Genital papillae are labelled using to the terminology of Sudhaus & Fürst von Lieven (2003), and laterally located papillae are indicated with "d".



**FIGURE 22.** Scanning electron photomicrographs of 12-flapped eurystomatous female of *Pristionchus magnoliae* **n. sp.** *en face* view with left and right amphids (la and ra) and left and right subventral (lsv and rsv), lateral (l) and left and right dorsal (ld and rd) labial sensillae, dorsal (dt) and right subventral (rsvt) teeth and dorsal gland orifice (dgo) indicated. Form type is not specified because the deeper part of the stoma is not seen.



**FIGURE 23.** Scanning electron photomicrograph of 12-flapped 'type 1' eurystomatous female of *Pristionchus magnoliae* **n. sp.** showing serrated anterior edge of pro-meso stegostom.



**FIGURE 24.** Scanning electron photomicrograph of *Pristionchus magnoliae* **n. sp.** male tail with the position of genital papilae according to the terminology of Sudhaus and Fürst von Lieven (2003).

#### Pristionchus musae n. sp.

urn:lsid:zoobank.org:act:E015F8AB-4F6F-46B1-A96A-85F724C7448F

**Etymology.** The species name is derived from the associated substrate from which the species was recovered. The strain was isolated from a rotting pseudostem of a plant of the banana family (*Musa* sp.) with soil material.

Measurements. See Table 4

Adult. General characters are as described above for the *triformis*-group.

Stenostomatous form. Cheilostom, gymnostom, pro-mesostegostom and telostegostom as described above. 12plated form was not found during observation. Arrangement of tooth and denticles in metastegostom as described above; dorsal movable tooth triangular, anterior end slightly curved, tooth often directed anteriorly; left subventral ridge with three minute, blunt or pointed adventitious denticles on a plate; right subventral ridge with three distal rounded or blunt adventitious denticles. Whole stoma including the tooth and denticles is narrow and more pointed in males.

Eurystomatous form. Cheilostom as described above, anterior half of each cheilostomal plate often split into two tips to form 12-flapped form. Six-flapped form (without split cheilostomal plate) rare. Gymnostom in both types 1 and 2 short and thick, forming cuticular ring. Pro-mesostegostom well-developed, internally overlapping with posterior end of gymnostom; type 1 form short, somewhat flattened and bearing weak serrates at anterior end; type 2 with small spines sparsely on inner surface. Arrangement of tooth and denticles in metastegostom as described above; dorsal movable tooth claw-like as typical of the genus; left subventral ridge with three large plates, each often has split tips and extra denticles on middle forming spiny plate; right subventral movable tooth claw-like and often bears extra peak on the ventral side. Telostegostom as described above.



**FIGURE 25.** *Pristionchus musae* **n. sp.** A: Right lateral view of adult female. B: Right lateral view of adult male. C: Anterior part of adult female in left lateral view. D: Body surface structure of deirid and postdeirid region showing the relative position of deirid and postdeirid where 'd' and 'v' indicates dorsal and ventral sides, respectively. E: Ventral view of secretory-excretory pore opening. F: Left lateral view of male head region showing labial sensilla, cephalic papillae and amphid. G, H: Stomal region of adult female in left lateral (G) and right lateral (H) view. I, J: Stomal region of eurystomatous female with pro-meso-stegostomatal spines (type 2) in left (I) and right (L) lateral view. K, L: Stomal region of eurystomatous female with pro-meso-stegostomatal serrates (type 1) in left (K) and right subventral ridge (right) (G), left subventral ridge (left) and dorsal tooth (middle and right) (H), right subventral tooth (left), left subventral ridge (middle) and dorsal tooth (right) (L), and dorsal (left and middle) and right subventral (right) teeth (L). M: Anterior female gonad in right lateral view. N: Vulval region in ventral view. O, P: Female tail in right lateral (O) and ventral (P) view. Q, R: Male tail in ventral (Q) and right lateral (R) view. S: Spicule and gubernaculum in right lateral view.



**FIGURE 26.** Stomal region of *Pristionchus musae* **n. sp.** A: Left lateral view of stenostomatous form in four different focal planes. B: Right lateral view of stenostomatous form in two different focal planes. C: Left lateral view of type 1 eurystomatous form in four different focal planes. D: Right lateral view of type 1 eurystomatous form.



**FIGURE 27.** Male tail characters of *Pristionchus musae* **n. sp.** Left lateral view of whole tail in three different focal planes and spicule and gubernaculum two different focal planes. Genital papillae are labelled using the terminology of Sudhaus & Fürst von Lieven (2003), and laterally located papillae are indicated with "d".



FIGURE 28. Scanning electron photomicrograph of vulval opening region of Pristionchus musae n. sp.

Male. Paired papillae and phasmid are arranged as <v1, v2d, v3, co, v4, ad, (ph, v5, v6, v7, pd)>, where v1 located about 1 CBD anterior to co; v2d less than 1/5 CBD anterior to co; v3 adcloacal; v4 at 1/3 CBD posterior to co, *i.e.*, v2d, v3, co and v4 are close to each other; ad about 1 CBD posterior to co; ph at 2/3 way from ad and the root of tail spike; v5-v7 forming triplet, just posterior to ph; and pd around level of v7, *i.e.*, ph, triplet papillae and pd are very close to each other. v1, v3, v4 and ph subventral, v2d and ad lateral, v5-7 ventral, pd subdorsal in male tail. General shape of spicule and gubernaculum as described above.

Female. Gonadal characters of female as described above. Tail elongate conoid with slightly filiform terminus, *i.e.*, the posterior half of tail is more elongated compared with anterior part. Phasmid ventro-laterally located at about 1.5 ABD posterior to anal opening.

**Diagnosis and relationships.** *Pristionchus musae* **n. sp.** is characterized by the slightly anteriorly curved triangular dorsal tooth and the right and left subventral ridges with three denticles of stenostomatous form, right subventral stegostomal tooth which often has blunt peak on the ventral side in eurystomatous form, sparsely serrated pro-mesostegostomal wall in type 2 eurystomatous form, the arrangement of male genital papillae, <v1, v2d, v3, co, v4, ad, (ph, v5, v6, v7, pd)> where v2d, v3, co and v4 and ph, v5, v6, v7, pd are close to each other, respectively, and a long spike of male tail. *Pristionchus musae* **n. sp.** is typologically close to *P. hoplostomus*. The new species and *P. hoplostomus* share a triangular and pointed dorsal tooth in stenostomatous form, right subventral tooth in eurystomatous form, often have an extra peak on its ventral side, and the arrangement of genital papillae, closely located v2d, v3, co and v4. The new species is distinguished from *P. hoplostomus* by the absence vs. presence of 12-plated stenostomatous form, and dorsal tooth of stenostomatous form, *i.e.*, the tooth is somewhat larger and more anteriorly directed in *P. hoplostomus* than *P. musae* **n. sp.** Further, the new species is distinguished from all other species by mating experiments and also characterized by a *ca.* 1,600-bp fragment of the SSU rRNA gene (GenBank accession number MW017220), the sequence of which is distinct from that of all other *Pristionchus* species.

TABLE 4.				
	P. magr	ioliae (RS5999)	P. mu	sae (RS5987)
character	stenostomatous male	stenostomatous female	stenostomatous male	stenostomatous female
n	5	5	5	5
L	$852 \pm 20.4 \ (822-877)$	$1011 \pm 89.3 \ (922-1111)$	$853 \pm 77.4 \ (749-949)$	$1015 \pm 84.7 \ (940-1120)$
Ľ	$689 \pm 12.9 \ (674-708)$	$778 \pm 77.8$ (708-879)	$688 \pm 65.2 \ (605-762)$	$787 \pm 59.5 \ (712-849)$
a	$14 \pm 0.9 (14-16)$	$13 \pm 0.8 \ (13-14)$	$16 \pm 1.4 \ (14-18)$	$16 \pm 1.1 \ (15-18)$
þ	$6.4 \pm 0.2 \ (6.2 - 6.8)$	$6.8 \pm 0.3 \ (6.4-7.2)$	$5.9 \pm 0.5 (5.3 - 6.6)$	$6.4 \pm 0.6 \ (5.6-7.3)$
c	$5.2 \pm 0.4 \; (4.8 - 5.7)$	$4.4 \pm 0.4 \ (4.0-4.9)$	$5.2 \pm 0.3 \ (4.7 - 5.6)$	$4.5 \pm 0.4 \ (4.1-5.0)$
c	$4.7 \pm 0.7 (3.8-5.6)$	$7.4 \pm 1.2 \ (6.3-9.2)$	$4.8 \pm 0.5 \ (4.2-5.3)$	$7.0 \pm 1.0 \ (5.9 - 8.1)$
ant. stoma length (cheilo- + gymnostom)	$7.3 \pm 0.4 \ (6.8-7.7)$	$8.4 \pm 0.9 (7.4-9.3)$	$7.8 \pm 0.8 \ (6.9 - 8.4)$	$8.7 \pm 0.5 \ (8.0-9.4)$
total stoma length	$11.5 \pm 0.3 (11.0-11.8)$	$14.1 \pm 0.4 \ (13.5 - 14.6)$	$12.3 \pm 0.7 (11.8 - 13.4)$	$13.8 \pm 0.7 (12.8 - 14.5)$
stoma width	$6.8 \pm 0.3 \ (6.4 - 7.1)$	$7.3 \pm 0.5 \ (6.6-7.8)$	$5.8 \pm 0.4 \ (5.5 - 6.5)$	$6.7 \pm 0.7$ (5.6-7.5)
ant. pharynx length (pro- + metacorpus)	$74 \pm 2.7$ (72-78)	$86 \pm 6.7 (78-93)$	$80 \pm 7.6$ (74-93)	$92 \pm 4.6 \; (86-99)$
post. pharynx length (isthmus + basal bulb)	$47 \pm 2.0$ (44-49)	$54 \pm 3.8 \ (49-59)$	$53 \pm 2.8 \ (50-57)$	58 ± 3.3 (54-61)
total length pharynx	$121 \pm 4.4 \ (117 - 127)$	$140 \pm 8.6 \ (129-150)$	$132 \pm 10.1 \ (125-150)$	$149 \pm 7.3 \ (140 - 160)$
ant./total pharynx %	$61 \pm 0.7 \ (60-62)$	$61 \pm 2.2 \ (58-64)$	$60 \pm 1.3 \ (59-62)$	$61 \pm 1.0 \ (60-63)$
median bulb diameter	$22 \pm 0.8 \ (21-23)$	$28 \pm 0.9 \ (26-28)$	$23 \pm 1.5 (22-25)$	27 ± 2.2 (23-29)
terminal bulb diameter	$19 \pm 0.9 \ (18-20)$	$24 \pm 1.5 \ (23-26)$	$19 \pm 1.3 \ (18-21)$	$23 \pm 1.4 \ (21-24)$
neck length (incl. stoma)	$132 \pm 4.5 \ (128-139)$	$148 \pm 9.3 \ (136-159)$	$145 \pm 10.6 \ (137 - 163)$	$158 \pm 7.3 \ (149-168)$
excretion pore	$134 \pm 6.3 \ (129-142)$	$149 \pm 10.9 (131 - 157)$	$141 \pm 10.2 \ (127 - 154)$	$149 \pm 9.7 (135 - 159)$
nerve ring	$95 \pm 2.9 \ (91-98)$	$111 \pm 7.0 \ (104-120)$	$107 \pm 7.8 \ (99-119)$	$119 \pm 4.2 \ (114-125)$
testis length	$475 \pm 13.9 \ (459-495)$		$458 \pm 61.8 \ (399-544)$	I
anterior gonad length	I	$190 \pm 34.2 \ (159-236)$	ı	$195 \pm 27.7 \ (174-241)$
posterior gonad length	ı	$156 \pm 18.7 \ (137-180)$	ı	$142 \pm 34.9 \ (104 - 194)$
ant. end to vulva distance	ı	$459 \pm 52.6 \ (410-530)$	ı	$468 \pm 33.8 \ (427-515)$
vulva to anus distance	I	$322 \pm 28.0 \ (301 - 359)$		$323 \pm 27.3 \ (289-361)$
T or V	$56 \pm 2.0 (52-58)$	$45 \pm 1.7 \ (44-48)$	$54 \pm 3.9 (50-60)$	$46 \pm 1.8 \ (43-48)$
max. body diameter	$59 \pm 2.6$ (56-62)	$76 \pm 2.8 \ (71-78)$	$55 \pm 6.6 (47-65)$	$64 \pm 7.2 (53-71)$
cloacal or anal body diameter	$35 \pm 3.0 (32-40)$	$32 \pm 2.4 \ (29-35)$	$35 \pm 2.5 \ (31 - 38)$	$33 \pm 2.4 \ (29-34)$
tail length	$163 \pm 14.3 \ (149-182)$	$233 \pm 23.7 \ (201-266)$	$166 \pm 16.3 \ (145-187)$	$229 \pm 32.5 \ (190-274)$
spicule length (curve)	$44 \pm 1.6 (42-45)$		$41 \pm 2.1 \ (39-44)$	I
spicule length (chord)	$33 \pm 2.5 \ (30 - 36)$		$33 \pm 2$ (30-35)	I
gubernaculum length	$16 \pm 0.8 \ (15 - 17)$		$15 \pm 0.5 (14-15)$	

Type host and locality. Pugao Laozhai village, close to road, Yuanyang, Yunnan province, PRC.

**Type material and type strain.** Type strain RS5987 frozen at the nematode collection of the MPI Tübingen and available as living culture upon request. Voucher specimens sent to the following museums: Holotype male, Para-type male and female: Museum für Naturkunde Karlsruhe, Germany; Paratype male and female: Swedish Natural History Museum, Stockholm, Sweden; Paratype male and female: University of California in Riverside Nematode Collection (UCRNC), Riverside, CA, USA.



FIGURE 29. Scanning electron photomicrograph of Pristionchus musae n. sp., showing anus (a) and phasmid (ph).

#### Pristionchus auriculatae n. sp.

urn:lsid:zoobank.org:act:57A7DF1B-E33B-4F73-8A6F-6A3ABCBC2600

**Etymology.** The species name is derived from the substrate from which the species was recovered. The type strain was isolated from a rotting fig of *Ficus auriculata* from soil substrate.

Measurements. See Table 5

Adult. General characters are as described above for the *triformis*-group.

Stenostomatous form. Cheilostom, gymnostom, pro-mesostegostom and telostegostom as described above. 12plated form not found. Arrangement of tooth and denticles in metastegostom as described above; dorsal movable tooth triangular, anterior end slightly curved, tooth sometimes directed anteriorly; left subventral ridge with three minute, blunt or pointed adventitious denticles on a plate; right subventral ridge with one or two distal rounded or blunt adventitious denticles. Whole stoma including the tooth and denticles is narrow and more pointed in males.

Eurystomatous form. Cheilostom as described above, anterior half of each cheilostomal plate often split into two tips to form 12-flapped form. Six-flapped form (without split cheilostomal plate) rare. Gymnostom in both types 1 and 2 short and thick, forming cuticular ring. Pro-mesostegostom well-developed, internally overlapping with posterior end of gymnostom; type 1 form short, somewhat flattened and bearing weak serrates at anterior end;



**FIGURE 30.** *Pristionchus auriculatae* **n. sp.** A: Right lateral view of adult female. B: Right lateral view of adult male. C: Anterior part of adult eurystomatous female in left lateral view. D: Left lateral view of male head region showing labial sensilla, cephalic papillae and amphid. E, F: Stomal region of adult female in left lateral (E) and right lateral (F) view. G, H: Stomal region of eurystomatous female with pro-mesostegostomatal serrates (type 1) in left (G) and right (H) lateral view. I, J: Stomal region of eurystomatous female with pro-mesostegostomatal spines (type 2) in left (I) and right (J) lateral view. Morphological variations of teeth and ridges are separately drawn in each subfigure as left subventral ridge (left) and dorsal tooth (right) (E), dorsal tooth (left) and right subventral widge (right) (F), left subventral ridge (left), and dorsal tooth (right) (G), dorsal (left) and right subventral (right) teeth (H), left subventral ridge (left) and dorsal (right) teeth (J).



**FIGURE 31.** *Pristionchus auriculatae* **n. sp.** A: Anterior female gonad in right lateral view. B, C: Female tail in left lateral (B) and ventral (C) view. D: Spicule and gubernaculum in left lateral view: E, F: Male tail in right lateral (E) and ventral (F) view.



**FIGURE 32.** Stomal region of *Pristionchus auriculatae* **n. sp.** A: Left lateral view of stenostomatous form in five different focal planes. B: Right lateral view of stenostomatous form in two different focal planes. C: Left lateral view of type 2 eurystomatous form in four different focal planes. D: Right lateral view of type 1 eurystomatous form in three different focal planes. E: Right lateral view of six-plated eurystomatous form in three different focal planes.



**FIGURE 33.** Male tail characters of *Pristionchus auriculatae* **n. sp.** A: Right lateral view of tail in four different focal planes. B: Spicule and gubernaculum three different focal planes. Genital papillae are labelled using the terminology of Sudhaus & Fürst von Lieven (2003), and laterally located papillae are indicated with "d".

type 2 with few small spines on inner surface. Arrangement of tooth and denticles in metastegostom as described above; dorsal movable tooth claw-like as typical of the genus; left subventral ridge with three large plates, each often has split tips and extra denticles on middle forming spiny plate; right subventral movable tooth claw-like and sometimes bears an extra peak on the ventral side. Telostegostom as described above.

TABLE 5.				
	P. auricu	tlatae (RS5989)	P. passali	dorum (RS6031)
character	stenostomatous male	stenostomatous male	stenostomatous male	stenostomatous female
n	5	5	5	5
L	$1076 \pm 92.3 \ (934 - 1188)$	$1076 \pm 92.3 \ (934 - 1188)$	$987 \pm 87.3 \ (893 - 1131)$	$1404 \pm 112.4 \ (1252 - 1532)$
Ľ	$898 \pm 85.8 \ (777 - 1000)$	$898 \pm 85.8 \ (777 - 1000)$	$791 \pm 88.3 \ (702 - 938)$	$1126 \pm 81.5 \ (1022 - 1217)$
а	$12 \pm 1.0 (11 - 13)$	$12 \pm 1.0 (11 - 13)$	$12 \pm 1.2 \ (10 - 13)$	$13 \pm 0.7 (12 - 14)$
þ	$6.6 \pm 0.5 \ (5.8 - 7.0)$	$6.6 \pm 0.5 \ (5.8 - 7.0)$	$6.3 \pm 0.5 \ (5.7 - 7.0)$	$7.9 \pm 0.4 \; (7.3 - 8.5)$
c	$6.1 \pm 0.7 \ (5.3 - 7.0)$	$6.1 \pm 0.7 \ (5.3 - 7.0)$	$5.1 \pm 0.6 \ (4.4 - 5.9)$	$5.1 \pm 0.2 \ (4.8 - 5.4)$
ç	$4.2 \pm 0.6 (3.7 - 5.1)$	$4.2 \pm 0.6 (3.7 - 5.1)$	$4.7 \pm 0.5 \ (3.9 - 5.1)$	$7.4 \pm 1.2 \ (5.9 - 8.7)$
ant. stoma length (cheilo- + gymnostom)	$8.7 \pm 0.5 \ (8.0 - 9.4)$	$8.7 \pm 0.5 \ (8.0 - 9.4)$	$9.4 \pm 0.7 \ (8.3 - 10.3)$	$10.3 \pm 1.1 \ (9.1 - 11.5)$
total stoma length	$13.7 \pm 0.5 \ (13.1 - 14.5)$	$13.7 \pm 0.5 \ (13.1 - 14.5)$	$14.3 \pm 0.5 \ (13.7 - 15.0)$	$15.3 \pm 1.6 \ (12.7 - 16.9)$
stoma width	$6.3 \pm 0.6 \ (5.5 - 7.0)$	$6.3 \pm 0.6 \ (5.5 - 7.0)$	$6.6 \pm 0.7 \ (5.8 - 7.6)$	$8.0 \pm 0.8 \; (7.2 - 9.0)$
ant. pharynx length (pro- + metacorpus)	$90 \pm 7.4 \ (84 - 103)$	$90 \pm 7.4 \ (84 - 103)$	$87 \pm 2.7 \ (84-91)$	$102 \pm 3.4 \ (99 - 108)$
post. pharynx length (isthmus + basal bulb)	$58 \pm 2.6 (56-62)$	$58 \pm 2.6 (56-62)$	57 ± 4.1 (52–62)	$65 \pm 4.6 \ (60-69)$
total length pharynx	$148 \pm 6.3 \ (142 - 159)$	$148 \pm 6.3 \ (142 - 159)$	$143 \pm 5.2 \ (139 - 151)$	$168 \pm 6.6 \ (161 - 177)$
ant./total pharynx %	$61 \pm 2.6 (58-65)$	$61 \pm 2.6 (58-65)$	$61 \pm 1.8 (58-63)$	$61 \pm 1.6 (59-63)$
median bulb diameter	$26 \pm 1.7 \ (24-28)$	$26 \pm 1.7 \ (24-28)$	$28 \pm 1.5 \ (27 - 30)$	$33 \pm 1.2 \ (32 - 35)$
terminal bulb diameter	$24 \pm 1.4 \ (22 - 26)$	$24 \pm 1.4 \ (22 - 26)$	$24 \pm 2.4 \ (21 - 27)$	$30 \pm 1.8 \ (27 - 32)$
neck length (incl. stoma)	$162 \pm 6.3 \ (156 - 172)$	$162 \pm 6.3 \ (156 - 172)$	$158 \pm 4.8 \ (153 - 164)$	$178 \pm 6.9 \ (172 - 189)$
excretion pore	$156 \pm 10.6 \ (141 - 168)$	$156 \pm 10.6 \ (141 - 168)$	152 ± 13.3 (142–175)	$172 \pm 14.4 \ (155 - 191)$
nerve ring	$112 \pm 2.4 \ (109 - 115)$	$112 \pm 2.4 \ (109 - 115)$	$116 \pm 4.5 \ (110 - 122)$	$125 \pm 10.9 (114 - 140)$
testis length	$660 \pm 66.6 \ (574-747)$	$660 \pm 66.6 (574 - 747)$	$552 \pm 68.4 \ (492 - 665)$	I
anterior gonad length	I	ı	I	$313 \pm 32.3 \ (286 - 363)$
posterior gonad length	I	ı	ı	$271 \pm 54.5 \ (233 - 366)$
ant. end to vulva distance	1		ı	$631 \pm 53.2 (573 - 691)$
vulva to anus distance	ı			$492 \pm 32.4 \ (451 - 536)$
T or V	$61 \pm 1.8 (59-63)$	$61 \pm 1.8 (59 - 63)$	$56 \pm 2.6 (52 - 59)$	$45 \pm 1.0 \ (43-46)$
max. body diameter	$88 \pm 9.1 \ (79-100)$	$108 \pm 5.7 \ (99-113)$	85 ± 9.2 (76–98)	$99 \pm 10.0 \ (83 - 108)$
cloacal or anal body diameter	$42 \pm 2.5 \ (40-46)$	$38 \pm 2.4 \; (34 - 40)$	$42 \pm 2.3 \ (40-44)$	$37 \pm 1.7 (35-40)$
tail length	$179 \pm 20.0 \ (158-203)$	279 ± 32.3 (230–315)	$196 \pm 16.6 \ (175-221)$	$301 \pm 16.5 \ (288 - 327)$
spicule length (curve)	$49 \pm 2.8 (44-51)$	ı	$43 \pm 1.2 \ (41 - 44)$	I
spicule length (chord)	$41 \pm 2.3 (38-43)$	ı	$36 \pm 0.7 \ (35 - 37)$	I
gubernaculum length	$18 \pm 0.8 \ (17 - 19)$		$17 \pm 0.7 (16 - 17)$	

Male. Paired papillae and the phasmid are arranged as <v1, v2d, v3, co, v4, ad, (ph, v5, v6, v7, pd)>, where v1 located about 1 CBD anterior to co; v2d just less than 1/5 CBD anterior to co; v3 adcloacal; v4 at 1/3 CBD posterior to co, *i.e.*, v2d, v3, co and v4 are close to each other; ad about 1 CBD posterior to co; ph 2/3 distance from ad and root of tail spike; v5–v7 forming triplet, just posterior to ph; and pd at level of triplet, *i.e.*, ph, triplet papillae and pd are close to each other. v1, v3, v4 and ph subventral, v2d and ad lateral, v5–7 ventral and pd subdorsal in male tail. General shape of spicule and gubernaculum as described above.

Female. Gonadal characters of female as described above. Tail elongate conoid with slightly filiform terminus, *i.e.*, posterior half of tail is more elongated compared to anterior part. Phasmid ventro-laterally located at about 1.5 ABD posterior to anal opening.

**Diagnosis and relationships.** *Pristionchus auriculatae* **n. sp.** is characterized by the slightly anteriorly curved triangular dorsal tooth and the right and left subventral ridges with three denticles of stenostomatous form, right subventral stegostomal tooth may have a blunt peak on the ventral side in eurystomatous form, sparsely serrated pro-mesostegostomal wall in type 2 eurystomatous form, the arrangement of male genital papillae, <v1, v2d, v3, co, v4, ad, (ph, v5, v6, v7, pd)> where v2d, v3, co and v4 and ph, v5, v6, v7, pd are close to each other, respectively, and a long spike occupying more than 2/3 of tail length of male tail. In addition, the arrangement of v1, v2d and ad genital papillae, where the distance between v1 and v2d is almost same as that between v2d and ad is characteristic to this species.

*Pristionchus auriculatae* **n. sp.** is typologically close to *P. hoplostomus*. The new species and *P. hoplostomus* share triangular and pointed dorsal tooth in stenostomatous form, right subventral tooth in eurystomatous form, sometimes have an extra peak on its ventral side, and the arrangement of genital papillae, closely located v2d, v3, co and v4 and pd overlapping with v5–7 triplet. The new species is distinguished from *P. hoplostomus* by the absence *vs.* presence of 12-plated stenostomatous form, right subventral ridge in stenostomatous form, with blunt *vs.* pointed denticles, and the arrangement of posterior four pairs of genital papillae, *i.e.*, pd is overlapping with triplet *vs.* posterior to v7.

Further, the new species is distinguished from all other species by mating experiments and also characterized by a *ca.* 1,600-bp fragment of the SSU rRNA gene (GenBank accession number MW017216), the sequence of which is distinct from that of all other *Pristionchus* species.

**Type host and locality.** Isolated from rotting *Ficus auriculata* fruits from soil substrates collected at the Botanical garden, Shanghai - conservatory 1, Shanghai, PRC.

**Type material and type strain.** Type strain RS5989 frozen at the nematode collection of the MPI Tübingen and available as living culture upon request. Voucher specimens sent to the following museums: Holotype male, Para-type male and female: Museum für Naturkunde Karlsruhe, Germany; Paratype male and female: Swedish Natural History Museum, Stockholm, Sweden; Paratype male and female: University of California in Riverside Nematode Collection (UCRNC), Riverside, CA, USA.

#### Pristionchus passalidorum n. sp.

urn:lsid:zoobank.org:act:DE5D7130-E1CE-4856-BD75-CAA18814BA00

**Etymology.** The species name is derived from the family name of the host, bess beetle (Coleoptera: Passalidae). **Measurements.** See Table 5

Adult. General characters are as described above for the *triformis*-group.

Stenostomatous form. Cheilostom, gymnostom, pro-mesostegostom and telostegostom as described above. 12plated form not found. Arrangement of tooth and denticles in metastegostom as described above; dorsal movable tooth triangular, anterior end slightly curved, tooth often directed anteriorly; left subventral ridge with three minute, blunt or pointed adventitious denticles on a plate; right subventral ridge with a distal pointed adventitious denticle. Whole stoma including tooth and denticles narrow and more pointed in males.

Eurystomatous form. Cheilostom as described above, anterior half of each cheilostomal plate often split into two tips to form 12-flapped form. Six-flapped form (without split cheilostomal plate) rare. Gymnostom short and thick in both types 1 and 2, forming cuticular ring. Pro-mesostegostom well-developed, internally overlapping with posterior end of gymnostom; type 1 form short flattened with weak serrates at anterior end; type 2 bearing few small spines on inner surface. Arrangement of tooth and denticles in metastegostom as described above; dorsal movable

tooth claw-like as typical of the genus; left subventral ridge with three large plates, each often has split tips and extra denticles on middle forming spiny plate; right subventral movable tooth claw-like often with extra peak on ventral side. Telostegostom as described above.



**FIGURE 34.** *Pristionchus passalidorum* **n. sp.** A: Right lateral view of adult female. B: Right lateral view of adult male. C: Left lateral view of male head region showing labial sensilla, cephalic papillae and amphid. D, E: Stomal region of adult female in left lateral (D) and right lateral (E) view. F, G: Stomal region of eurystomatous female with pro-mesostegostomatal serrates (type 1) in left (F) and right (G) lateral view. H, I: Stomal region of eurystomatous female with pro-mesostegostomatal spines (type 2) in left (H) and right (I) lateral vie. Morphological variations of teeth are separately drawn in each subfigure as left subventral ridge (left and middle) and dorsal tooth (right) (D), dorsal tooth (left) and right subventral ridge (right) (E), left subventral ridge (left), and dorsal tooth (right) (F), dorsal (left) and right subventral (right) teeth (G), left subventral ridge (left and middle) and dorsal (left and middle) and right subventral (right) teeth (I). J: Anterior part of adult eurystomatous female in right lateral view. K: Relative position of deirid and secretory-excretory pore of eurystomatous female. L: Anterior part of adult stenostomatous male in right lateral view. M: Relative position of deirid and secretory-excretory pore of stenostomatous male.



**FIGURE 35**. *Pristionchus passalidorum* **n. sp.** A: Male tail in left lateral (A) and ventral (B) views. C: Spicule and gubernaculum in left lateral view. D: Anterior female gonad in right lateral view. E: Vulval region in ventral view. F, G: Female tail in right lateral (F) and ventral (G) views.

Male. Paired papillae and phasmid are arranged as <v1, v2d, v3, co, v4, ad, (ph, v5, v6, v7, pd)>, where v1 located about 1 CBD anterior to co; v2d just less than 1/5 CBD anterior to co; v3 adeloacal; v4 at 1/3 CBD posterior to co, *i.e.*, v2d, v3, co and v4 are close to each other; ad about 1 CBD posterior to co; ph 2/3 distance from ad and rest of tail spike; v5-v7 forming triplet, just posterior to ph; and pd around the level of v7, *i.e.*, ph, triplet papillae and pd close to each other. v1, v3, v4 and ph subventral, v2d and ad lateral, v5-7 ventral, pd subdorsal in the male tail. General shape of spicule and gubernaculum as described above.

Female. Gonadal characters of female as described above. Tail elongate conoid with slightly filiform terminus, *i.e.*, the posterior half of tail is more elongated compared to anterior part. Phasmid ventro-laterally located at about 1.5 ABD posterior to anal opening.

**Diagnosis and relationships.** *Pristionchus passalidorum* **n. sp.** is characterized by the slightly anteriorly curved triangular dorsal tooth, the right and left subventral plates with one and three denticle(s) in stenostomatous form, right subventral stegostomal tooth without extra peak in eurystomatous form, sparsely serrated pro-mesostegostomal wall in type 2 eurystomatous form, and the arrangement of male genital papillae, <v1, v2d, v3, co, v4, ad, (ph, v5, v6, v7, pd)> where v2d, v3, co and v4 and ph, v5, v6, v7, pd are close to each other, respectively, and a long spike occupying more than 2/3 of tail length of male tail. *Pristionchus passalidorum* **n. sp.** is typologically similar to *P. hoplostomus* and *P. musae* **n. sp.** The new species and these two species share a triangular and pointed dorsal

tooth with slightly anteriorly curved tip in stenostomatous form and the arrangement of genital papillae, closely located v2d, v3, co and v4. The new species is distinguished from *P. hoplostomus* by the absence vs. presence of 12-plated stenostomatous form, left subventral ridges of stenostomatous form, clearly pointed vs. bluntly pointed, right subventral plate of stenostomatous form, single vs. multiple peaks, right subventral tooth of eurystomatous form, without vs. with extra peak. The new species is distinguished from all other species by mating experiments and also characterized by a *ca*. 1,600-bp fragment of the SSU rRNA gene (GenBank accession number MW017223), the sequence of which is distinct from that of all other *Pristionchus* species.

**Type host and locality.** Bessbug (Coleoptera: Passalidae) at Ailaoshan Field Station CAS, Yunnan province, PRC.

**Type material and type strain.** Type strain RS6031, frozen at the nematode collection of the MPI Tübingen and available as living culture upon request. Voucher specimens sent to the following museums: Holotype male, Paratype male and female: Museum für Naturkunde Karlsruhe, Germany; Paratype male and female: Swedish Natural History Museum, Stockholm, Sweden; Paratype male and female: University of California in Riverside Nematode Collection (UCRNC), Riverside, CA, USA.



**FIGURE 36.** Stomal region of *Pristionchus passalidorum* **n. sp.** A: Surface of stenostomatous female in left lateral view. B: Dorsal tooth and left subventral ridge of stenostomatous in left lateral view. C: Dorsal tooth and right subventral ridge of stenostomatous form in right lateral view Right lateral view of stenostomatous form in two different focal planes. D: Left lateral view of type 2 eurystomatous form in four different focal planes. E: Right lateral view of type 1 eurystomatous form in four different focal planes.



**FIGURE 37.** *Pristionchus passalidorum* **n. sp.** A: Right lateral view of posterior pharynx region in three different focal planes where deirid (d), lateral field (lf), nerve ring (nr), median bulb (mb) secretory-excretory pore (ep) and basal bulb (bb) are suggested. B: Left lateral view of postdeirid region in three different focal planes where postdeirid (pd) and its internal connection (ic), lateral gland opening (lg) and its associated secretory cell (sc) and posterior gonad (pg) are suggested. C: Female anal region in three different focal planes where lateral gland and its associated secretory cell, phasmid (ph) and rectal glands (rg) are indicated.



**FIGURE 38.** Male tail characters of *Pristionchus passalidorum* **n. sp.** Left lateral view of tail in two different focal planes and spicule and gubernaculum four different focal planes. Genital papillae are labelled using the terminology of Sudhaus & Fürst von Lieven (2003), and laterally located papillae are indicated with "d".



**FIGURE 39.** Scanning electron photomicrograph of *Pristionchus passalidorum* **n. sp.** stenostomatous male *en face* view where left and right amphids (la and ra), left and right subventral (lsv and rsv), left and right lateral (l and r) and left and right dorsal (ld and rd) labial sensilla, and left and right subventral (lsvc and rsvc) and dorsal (ldc and rdc) cephalic sensilla are indicated.



**FIGURE 40.** Scanning electron photomicrograph of *Pristionchus passalidorum* **n. sp.** male tail with the position of genital papillae shown according to the terminology of Sudhaus and Fürst von Lieven (2003).

# Species description of P. dorci n. sp. and P. purgamentorium n. sp. of the Pristionchus maupasi-group

Adult. Body cylindrical, stout, *i.e.*, body length-maximum body diam. ratio (a value) is usually ranging from 10–14, depending on culture condition; cuticle thick with fine annulation and longitudinal striations. Head without apparent lips, six mound-like anteriorly directed expansions, one on each sector. Six labial sensilla, one on each lip sector (on the mound); four small papilliform cephalic papillae in males on right and left subventral and right and left dorsal sectors; amphidial apertures located on lateral sector, slightly dorsally shifted, at level of margin of cheilo- and gymnostom. Stomal dimorphism present, and detailed morphology of the stoma is described below. Dorsal pharyngeal gland clearly observed, penetrating dorsal tooth to gland opening. Anterior pharynx longer than posterior pharynx; procorpus muscular, stout, occupying half to two-thirds of corresponding body diameter; metacorpus muscular, forming median bulb; isthmus narrow, not muscular; basal bulb glandular. Pharyngo-intestinal junction (cardia) clearly observed, well-developed. Intestine simple tube, not forming pre-rectum, extended posteriorly from cardia to rectum; three (two subventral and one dorsal) rectal gland cells observed at distal end of intestine (margin between intestine and rectum). Secretory-excretory pore not conspicuous, ventrally located at level of isthmus to pharyngo-intestinal junction, excretory duct extending anteriad and reflexed back to position of pore; two large secretory-excretory cells around the excretory duct. Deirid observed laterally on lateral field, located at the level around the posterior end of basal bulb to pharyngo-intestinal junction to a half body diameter posterior to the junction, ca. 0.5-1 body diam. posterior to secretory-excretory pore. Hemizonid not observed. Lateral glands (small pores connected to secretory cell) on lateral body surface, with positions inconsistent among individuals, numbering 5 to 8 for males and 9 to 13 for females. Postdeirid at anterior part of vas deferens in male and the posterior end of posterior gonad in female, on the same striation with deirid (= lateral field) or on the adjacent striation or the second dorsally neighboring striation to lateral field.

Stenostomatous form. Cheilostom consisting of six per- and interradial plates. Incision between plates not always distinguished. Anterior end of each plate rounded and elongated to project from stomaal opening and form a small flap. Split cheilostomal plate not observed. Gymnostom short, cuticular ring-like anterior end overlapping cheilostom internally; dorsal gymnostomal wall with mound-like expansion probably derived from metastegostom which gives an appearance of thickened dorsal gymnostomal wall compared to ventral side. Stegostom separated into three subsections: pro-meso, meta, and telostegostom. Pro-mesostegostom forming a weakly cuticularized ring internally overlapping with gymnostoma to connect gymnostom and metastegostom. Metastegostom bearing 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; pointed left subventral ridge with three minute adventitious denticles on a plate, most ventral denticle often masked by remaining two in lateral view; pointed right subventral ridge, often with distinct distal adventitious denticle(s). Telostegostom forming weakly sclerotized cup-like cavity connecting stoma and pharynx. Whole stoma including tooth and denticles narrow and more pointed in males.

Eurystomatous form. Cheilostom divided into six well-distinctive per- and interradial plates. Anterior end of each plate rounded and elongated to stick out from stomal opening and form a small flap. Split cheilostomal plate not observed. Gymnostom with thick cuticle, forming short, ring-like tube with more heavily sclerotized wall in the posterior; anterior end of gymnostom internally overlapping posterior end of cheilostomatal plates; lacking the serrates at anterior end of gymnostom. Pro-mesostegostom forming a weakly cuticularized ring connecting gymnostom and metastegostom. Metastegostom bearing large claw-like dorsal tooth; claw-like right subventral tooth; and three left subventral denticles, where the tip of each denticle sometimes splits into two or three small ridges. Telostegostom forming weakly sclerotized cup-like cavity connecting stoma and pharynx.

Male. Whole body ventrally arcuate, strongly ventrally curved at tail region when killed by heat. Testis single, ventrally located, anterior part reflexed to right or left side; spermatogonia arranged in three to five rows in reflexed part, well-developed spermatocytes arranged as three to four rows in anterior two-thirds of main branch, mature amoeboid spermatids arranged in multiple rows in proximal part of gonad. Vas deferens not clearly separated from other parts of gonad. Posterior end of vas deferens and rectum fused to form a cloacal tube. Spicules paired, separate; spicules smoothly curved in ventral view, adjacent to each other for distal third of their length, each smoothly tapering to pointed distal end; spicule in lateral view smoothly ventrally arcuate, giving spicule about 100° curvature, oval manubrium at anterior end, lamina/calomus complex smoothly tapering to pointed distal end. Gubernaculum conspicuous, about one-third of spicule length, broad anteriorly such that dorsal wall is slightly recurved with dorsal and ventral walls separate at 40–50° angle at posterior end; dorsal side of gubernaculum possessing single, membranous, anteriorly directed process and lateral pair of more sclerotized, anteriorly and obliquely ventrally directed processes. In lateral view, anterior half of gubernaculum with two successive curves separated by anteriorly and obliquely ventrally directed process, with anterior terminal curvature highly concave and almost closed, with deep posterior curvature being one-third of gubernaculum length; posterior half forming tube-like process enveloping spicules. Cloacal opening (co) slit-like in ventral view; one small, ventral, single genital papilla (vs) on anterior cloacal lip. All nine paired genital papillae papilliform. Paired papillae and phasmid arranged as <v1, v2, v3d, co, v4, ad, ph, (v5, v6, v7, pd)>, where v1 located about 1.5 CBD anterior to co; v2 about 0.5 CBD anterior to co; v3d less than 1/10 CBD posterior to v2, i.e., about 1/3 CBD anterior to co; v4 at about 1/3 CBD posterior to co; ad about 1 CBD posterior to co; ph midway between ad and root of tail spike; v5–v7 forming triplet, just posterior to ph; and pd level of ventral triplet (v5–v7). v1, v2, v4 and ph subventral, v3d and ad lateral, v5–7 ventral and pd subdorsal in the male tail. General shape of spicule and gubernaculum as described above, spicule relatively thin, gubernaculum relatively low (flattened). Male tail spike long.

Female. Body relaxed or weakly ventrally arcuate when killed by heat. Gonad didelphic, amphidelphic; each gonadal system arranged from vulva/vagina as uterus, oviduct, and ovary; anterior gonad right of intestine, with uterus and oviduct extending ventrally and anteriorly on right of intestine and with totally reflexed (=antidromous reflexion) ovary extending dorsally on left of intestine; oocytes mostly arranged in three to four or more 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 end of oviduct (=junction tissue between ovary and oviduct) consists of rounded cells; anterior part of oviduct consists of rounded cells, forming a simple tube; middle part of oviduct serving as spermatheca, consists of roundish and relatively large cells. Eggs in single to multiple-cell stage or even further developed at posterior part of oviduct (=uterus), in young females being composed of squared or angular cells, long

enough to contain one well-developed oocyte. *Receptaculum seminis* not observed, *i.e.*, the organ is not independent, and a part of oviduct/uterus works as the organ; vaginal glands present but obscure; vagina perpendicular to body surface, surrounded by sclerotized tissue; vulva slightly protuberant in lateral view, pore-like in ventral view; rectum about one anal body diameter (ABD) long, intestine/rectum junction surrounded by well-developed sphincter muscle. Anus in form of dome-shaped slit, posterior anal lip slightly protuberant. Tail elongate conoid with long and filiform terminus. Phasmid ventro-laterally located at about 1.5–2.0 ABD posterior to anal opening.

#### Pristionchus dorci n. sp.

urn:lsid:zoobank.org:act:55E69BA4-6D38-48A3-9D3F-A73D7E3F5E5A

**Etymology.** The species name is derived from the generic name of the host Lucanid beetle (*Dorcus davidis* [Fair-maire, 1887]).

## Measurements. See Table 6

Adult. General characters are as described above for maupasi-group.

Stenostomatous form. Dorsal movable tooth flint-shaped with anterior end slightly curved; left subventral ridge with three minute, blunt or pointed and adventitious denticles on a plate, most dorsal denticle often masked by middle denticle; right subventral ridge with a distal pointed adventitious denticle.

Eurystomatous form. Dorsal movable tooth claw-like as typical of the genus; left subventral ridge with three large plates, often with two or three split tips; right subventral movable tooth claw-like and extra peak not observed.

Male. As described above for *maupasi*-group.

Female. As described above for *maupasi*-group.

**Diagnosis and relationships.** *Pristionchus dorci* **n. sp.** is characterized by the slightly anteriorly curved flintshaped dorsal tooth, the left subventral ridge with three pointed or blunt denticles and right subventral ridge with a small pointed denticle of stenostomatous form, claw-like right subventral stegostomal tooth without an extra peak in eurystomatous form, the arrangement of male genital papillae, <v1, v2, v3d, co, v4, ad, ph, (v5, v6, v7, pd)> where posterior four paired papillae are forming cluster, and a long tail of both sexes. The maupasi-group of Pristionchus currently contains 15 species. These species are typologically similar with each other, but can be distinguished by the stomatal characters, e.g., number of denticles, and the arrangement of genital papillae (summarized in Kanzaki et al. 2018). P. dorci n. sp. shares its single-peaked right subventral ridge in the stenostomatous form and the laterally directed third paired male genital papillae (v3d) with P. laevicollis Kanzaki, Herrmann, Yoshida, Weiler, Rödelsperger & Sommer, 2018, P. japonicus Kanzaki, Ragsdale, Herrmann, Mayer & Sommer, 2012 and P. hongkongensis Kanzaki, Herrmann, Yoshida, Weiler, Rödelsperger & Sommer, 2018, which have single-peaked right subventral ridge and v3d papillae. The new species is also similar to P. maxplancki Kanzaki, Ragsdale, Herrmann, Röseler & Sommer, 2013 and P. quartusdecimus Kanzaki, Ragsdale, Herrmann, Röseler & Sommer, 2013 which has 1-2 peaks on the right subventral ridge and v3d papillae. However, P. dorci n. sp. is distinguished from P. laevicollis by the relative position of genital papillae, v2 and v3d two pairs are close to each other vs. clearly separated, the v3d is at 1/3 CBD anterior to co vs. very close to co and sometimes adcloacal, and the ventral triplet papillae and pd overlap each other vs. pd is located at just posterior to the triplet papillae. P. dorci n. sp. is distinguished from P. japonicus by the relative position of genital papillae, v1 is at 1.5 CBD vs. 1.0 CBD anterior to co, v1-v2 distance is almost same as vs. obviously shorter than v2–v4 distance, and female tail elongated conoid with vs. without filiform terminus. The new species is also readily distinguished from *P. hongkongensis* by the relative position of genital papillae, v1 is at 1.5 CBD vs. 1 CBD anterior to co, v1-v2 equivalent vs. shorter than v2-v4 distance, and the spicule shape, the spicule of P. dorci n. sp. is slenderer than that of P. hongkongensis. In addition, P. hongkongensis is characterized by its stomal morphology of eurystomatous form, *i.e.*, large and barrel-shaped stoma bearing left subventral ridges with many cusps, and this character is clearly different from the stomal morphology of new species. P. dorci n. sp. is distinguished from *P. quartusdecimus* by the right subventral ridge of stenostomatous form, with single vs. 1-2 peaks, and relative position of genital papillae, v1 is at 1.5 CBD vs. 1.0 CBD anterior to co, v1-v2 distance is almost same as vs. obviously shorter than  $v_{2}-v_{4}$  distance, and the ventral triplet papillae and pd overlap to each other vs. pd is located at just posterior to the triplet papillae. Typological characters, e.g., the arrangement of genital papillae, of new species is very similar to those of P. maxplancki. However, P. dorci n. sp. is distinguished from P. maxplancki by the right subventral ridge of stenostomatous form, with single vs. 1-2 peaks, and the relative position of posterior four paired papillae, the ventral triplet papillae and pd overlap to each other *vs.* pd is located at just posterior to the triplet papillae. Further, the new species is distinguished from all other species by mating experiments and also characterized by a *ca.* 1,600-bp fragment of the SSU rRNA gene (GenBank accession number MW017218), the sequence of which is distinct from that of all other *Pristionchus* species.



**FIGURE 41.** *Pristionchus dorci* **n. sp.** A: Right lateral view of adult male. B: Right lateral view of adult female. C: Left lateral view of male head region showing labial sensilla, cephalic papillae and amphid. D, E: Stomal region of adult female in left lateral (D) and right lateral (E) view. F, G: Stomal region of eurystomatous female in left (F) and right (G) lateral view. Morphological variations of teeth and ridges are separately drawn in each subfigure as left subventral ridge (left) and dorsal tooth (right) (D), dorsal tooth (left) and right subventral ridge (right) (E), left subventral ridge (left) and dorsal tooth (right) (F), and dorsal (left) and right subventral (right) teeth (G). H: Anterior part of adult female in left lateral view.



**FIGURE 42.** *Pristionchus dorci* **n. sp.** A: Anterior gonad of female in right lateral view. B: Vulval region in ventral view. C, D: Female tail in ventral (C) and left lateral (D) view. E, F: Male tail in left lateral (E) and ventral (F) view. G: Spicule and gubernaculum in left lateral view.

**Type host and locality.** Isolated from a specimen of the lucanid beetle *Dorcus davidis* collected at a dumpster near Ganquan, Shasnxi province, PRC.

**Type material and type strain.** Type strain RS 6134, other strain RS5992; frozen at the nematode collection of the MPI Tübingen and available as living culture upon request. Voucher specimens sent to the following museums: Holotype male, Paratype male and female: Museum für Naturkunde Karlsruhe, Germany; Paratype male and female: Swedish Natural History Museum, Stockholm, Sweden; Paratype male and female: University of California in Riverside Nematode Collection (UCRNC), Riverside, CA, USA.



**FIGURE 43.** Stomal region of *Pristionchus dorci* **n. sp.** A: Left lateral view of stenostomatous form in five different focal planes. B: Right lateral view of stenostomatous form in two different focal planes. C: Left lateral view of eurystomatous form in three different focal planes. D: Right lateral view of eurystomatous form in two different focal planes.



**FIGURE 44.** *Pristionchus dorci* **n. sp.** A: Right lateral view of posterior pharynx region in five different focal planes where deirid (d), lateral gland opening (lg) and its associated secretory cell (sc), nerve ring (nr), secretory-excretory pore (ep) and basal bulb (bb) are indicated. B: Left lateral view of female anal region in two different focal planes where phasmid (ph) and rectal glands (rg) are indicated. C: Right lateral view of whole tail in two different focal planes and spicule and gubernaculum in two different focal planes. Genital papillae are labelled using the terminology of Sudhaus & Fürst von Lieven (2003), and laterally located papillae are indicated with "d".



**FIGURE 45.** Scanning electron photomicrograph of *Pristionchus dorci* **n. sp.** surface structure with deirid (d) and lateral galnd (lg) indicated.



**FIGURE 46.** Scanning electron photomicrograph of *Pristionchus dorci* **n. sp.** stenostomatous male *enface* view where left and right amphids (la and ra), left and right subventral (lsv and rsv), left and right lateral (l and r) and left and right dorsal (ld and rd) labial sensilla, and right subventral (rsvc) and dorsal (ldc and rdc) cephalic sensilla are indicated. Left subventral cephalic sensillum is not seen.



**FIGURE 47.** Scanning electron photomicrograph of *Pristionchus dorci* **n. sp.** male tail with the position of genital papillae according to the terminology of Sudhaus and Fürst von Lieven (2003).

#### Pristionchus purgamentorium n. sp.

#### urn:lsid:zoobank.org:act:EE7F188B-438B-4E11-BF8E-5D389514932A

**Etymology.** The species name is derived from the finding circumstances. The beetle was collected on a dumpster (lat. purgamentum = trash).

Measurements. See Table 6

Adult. General characters are as described above for *maupasi*-group.

Stenostomatous form. Dorsal movable tooth flint-shape, anterior end slightly curved; left subventral ridge with three or sometimes more minute pointed and adventitious denticles on a plate, most dorsal denticle often masked by middle denticle; right subventral ridge with two or three bluntly pointed adventitious denticles.

Eurystomatous form. Dorsal movable tooth claw-like as typical of the genus; left subventral ridge with three large plates, each often has two or three split tips; right subventral movable tooth claw-like, an indistinctive extra peak sometimes seen.

Male. As described above for *maupasi*-group.

Female. As described above for *maupasi*-group.

**Diagnosis and relationships.** *Pristionchus purgamentorium* **n. sp.** is characterized by the slightly anteriorly curved flint-shaped dorsal tooth, the left subventral ridge with three or sometimes more pointed denticles and right subventral ridge with two or three small bluntly pointed denticles of stenostomatous form, claw-like right subventral stegostomal tooth sometimes with an indistinctive extra peak in eurystomatous form, the arrangement of male genital papillae, <v1, v2, v3d, co, v4, ad, ph, (v5, v6, v7, pd)> where posterior four paired papillae form a cluster,



**FIGURE 48.** *Pristionchus purgamentorium* **n. sp.** A: Right lateral view of adult female. B: Right lateral view of adult male. C: Left lateral view of male head region showing labial sensilla, cephalic papillae and amphid. D, E: Stomal region of adult female in left lateral (D) and right lateral (E) view. F, G: Stomal region of eurystomatous female in left (F) and right (G) lateral view. Morphological variations of teeth and ridges are separately drawn in each subfigure as left subventral ridge (left and middle) and dorsal tooth (right) (D), dorsal tooth (left) and right subventral ridge (middle and right) (E), left subventral ridge (left and middle) and dorsal tooth (right) (F), and dorsal (left) and right subventral (middle and right) teeth (G). H: Anterior part of adult stenostomatous female in left lateral view; I: relative position of secretory-excretory pore, deirid and lateral glands.



**FIGURE 49.** *Pristionchus purgamentorium* **n. sp.** A, B: Male tail in right lateral (E) and ventral (F) views. C: Spicule and gubernaculum in right lateral view. D: Anterior gonad of female in right lateral view. E: Vulval region in ventral view. F, G: Female tail in ventral (C) and right lateral (D) views.

and distinctive and long tail of both sexes. The *maupasi*-group currently contains 16 species including *P. dorci* **n. sp.**. These species are typologically similar to each other, but can be distinguished by the stomatal characters, *e.g.*, number of denticles, and the arrangement of genital papillae (summarized in Kanzaki *et al.*, 2018). *Pristionchus purgamentorium* **n. sp.** shares its multiple-peaked right subventral plate in the stenostomatous form and the laterally directed third paired male genital papillae (v3d) with *P. maxplancki*, which have one to two-peaked right subventral ridge and v3d papillae. The new species also shares multiple-peaked right subventral plate of stenostomatous form with *P. riukiariae* Kanzaki, Herrmann, Yoshida, Weiler, Rödelsperger & Sommer, 2018. However, *P. purgamentorium* **n. sp.** is distinguished from *P. maxplancki* by its right subventral plate of stenostomatous form, with two to three ridges *vs.* one to two ridges. *P. purgamentorium* **n. sp.** is also distinguished from *P. riukiariae* by right subventral plate of stenostomatous form, with two to three ridges *vs.* two ridges, and the arrangement of genital papillae, third *vs.* second pair directed laterally (v3d *vs.* v2d), and second and third pairs are separated *vs.* very close to each other. Further, the new species is distinguished from all other species by mating experiments and also characterized by a *ca.* 1,600-bp fragment of the SSU rRNA gene (GenBank accession number MW017224), the sequence of which is distinct from that of all other *Pristionchus* species.



**FIGURE 50.** Stomal region of *Pristionchus purgamentorium* **n. sp.** A: Left lateral view of stenostomatous form in four different focal planes. B: Right lateral view of stenostomatous form in three different focal planes. C: Left lateral view of eurystomatous form in four different focal planes. D: Right lateral view of eurystomatous form in two different focal planes.



**FIGURE 51.** *Pristionchus purgamentorium* **n. sp.** A: Right lateral view of posterior pharynx region in three different focal planes where deirid (d), lateral gland opening (lg) and its associated secretory cell (sc) and secretory-excretory pore (ep) are labelled. B: Right lateral view of male tail in five different focal planes. C: Ventral view of male tail in three different focal planes. Genital papillae are labelled according to the terminology of Sudhaus & Fürst von Lieven (2003), and laterally located papillae are indicated with "d".

TABLE 6.				
	P. dorc	i (RS6134)	P. purgamen	torium (RS6138)
character	stenostomatous male	stenostomatous female	stenostomatous male	stenostomatous female
n	5	5	5	5
L	$973 \pm 91.4 \ (874 - 1105)$	$1126 \pm 109.9 \ (1023 - 1313)$	$688 \pm 51.2 \ (648 - 768)$	$905 \pm 52.7 \ (844-985)$
L'	799 ± 87.7 (707–912)	$870 \pm 88.2 \ (792 - 1021)$	542 ± 47.2 (501–615)	$686 \pm 57.0 \ (633-773)$
3	$12 \pm 1.8 \ (10 - 14)$	$14 \pm 0.5 \ (13-14)$	$13 \pm 1.0 \ (12 - 14)$	$12 \pm 0.2 \ (12 - 12)$
b	$6.2 \pm 0.6 \ (5.7 - 7.2)$	$7.4 \pm 0.7 \ (6.6 - 8.6)$	$5.1 \pm 0.2 \ (5.0 - 5.4)$	$6.1 \pm 0.3 \ (5.7 - 6.5)$
C	$5.6 \pm 0.8 \ (4.7 - 6.9)$	$4.4 \pm 0.1 \ (4.2 - 4.5)$	$4.7 \pm 0.2 \ (4.4 - 5.0)$	$4.2 \pm 0.5 (3.4 - 4.6)$
C,	$4.1 \pm 0.5 (3.3 - 4.5)$	$7.1 \pm 0.6 \ (6.6 - 8.1)$	$4.2 \pm 0.2 \ (4.0 - 4.4)$	$6.7 \pm 1.1 \ (4.9 - 7.5)$
ant. stoma length (cheilo- + gymnostom)	$7.5 \pm 0.8 \ (6.9 - 8.6)$	$7.3 \pm 1.0 \ (6.0 - 8.7)$	$6.4 \pm 0.6 \ (5.7 - 7.1)$	$8.0 \pm 0.6 \; (7.4 - 9.0)$
total stoma length	$11.2 \pm 0.4 \ (10.7 - 11.7)$	$12.5 \pm 0.9 \ (11.6 - 13.7)$	$10.5 \pm 1.1 \ (9.6 - 12.3)$	$13.0 \pm 0.8 \ (12.1 - 14.2)$
stoma width	$7.0 \pm 0.5 \ (6.2 - 7.5)$	$7.7 \pm 1.0 \ (6.4 - 8.9)$	$5.2 \pm 0.3 \ (4.8 - 5.6)$	$6.3 \pm 0.9 (5.0 - 7.3)$
ant. pharynx length (pro- + metacorpus)	83 ± 3.2 (78–87)	$86 \pm 4.1 \ (83-93)$	78 ± 2.0 (76–80)	87 ± 3.4 (84–91)
post. pharynx length (isthmus + basal bulb)	$62 \pm 4.4 (55-66)$	$59 \pm 3.4 (55-62)$	$46 \pm 4.4 \; (41 - 51)$	$54 \pm 3.5 (51 - 59)$
total length pharynx	$145 \pm 3.3 \ (142 - 150)$	$145 \pm 2.9 \ (141 - 149)$	$124 \pm 5.1 \ (119 - 131)$	$142 \pm 5.1 \ (135 - 148)$
ant./total pharynx %	57 ± 2.5 (55–61)	$59 \pm 2.3 (57-63)$	$63 \pm 2.2 \ (60-66)$	$62 \pm 1.7 \ (59-64)$
median bulb diameter	$29 \pm 1.2 \ (28-31)$	27 ± 2.9 (25–32)	$26 \pm 1.3 \ (25-29)$	$31 \pm 1.3 \ (29 - 33)$
terminal bulb diameter	$26 \pm 2.6 \ (25 - 31)$	23 ± 2.8 (20–27)	$22 \pm 1.2 \ (21 - 23)$	$28 \pm 1.6 \ (26 - 30)$
neck length (incl. stoma)	$156 \pm 3.2 \ (153 - 161)$	$153 \pm 2.6 \ (149 - 155)$	$134 \pm 5.7 \ (130 - 143)$	$150 \pm 5.2 \ (143 - 156)$
excretion pore	$147 \pm 14.2 \ (129 - 167)$	$148 \pm 7.9 \ (142 - 161)$	$112 \pm 9.5 \ (102 - 123)$	$122 \pm 8.5 \ (111 - 135)$
nerve ring	$106 \pm 6.1 \ (95 - 110)$	$111 \pm 4.4 \ (103 - 114)$	$96 \pm 4.8 \; (91 - 103)$	$108 \pm 2.9 \ (104 - 111)$
testis length	$483 \pm 72.4 \ (398-577)$		$344 \pm 40.4 \ (308-410)$	
anterior gonad length	ı	$188 \pm 27.8 \ (149-224)$		$163 \pm 34.1 \ (135-222)$
posterior gonad length	ı	$214 \pm 35.0 \ (187 - 274)$		$144 \pm 12.4 \ (127 - 158)$
ant. end to vulva distance	ı	$503 \pm 47.3 \ (452 - 581)$		$414 \pm 37.4 (376 - 475)$
vulva to anus distance		$368 \pm 36.8 \ (344-432)$		$271 \pm 29.3 (223 - 293)$
T or V	49 ± 2.9 (46−52)	$45 \pm 0.6 (44-45)$	$50 \pm 2.6 \; (47 - 53)$	$46 \pm 1.6 \ (45-48)$
max. body diameter	$84 \pm 10.0 \ (72-94)$	$82 \pm 11.0$ (73–101)	$54 \pm 6.0 \ (46-61)$	75 ± 4.7 (70–81)
cloacal or anal body diameter	$43 \pm 4.2 \ (37 - 49)$	$36 \pm 4.9 \ (31 - 44)$	$35 \pm 1.6 \ (33 - 37)$	$34 \pm 6.0 \ (28-43)$
tail length	$174 \pm 20.9 \ (142 - 193)$	$256 \pm 22.3 \ (231 - 292)$	$147 \pm 5.2 \ (139 - 153)$	$220 \pm 24.0 \ (201 - 261)$
spicule length (curve)	59 ± 2.0 (57−62)		$43 \pm 1.8 \ (41 - 45)$	
spicule length (chord)	$48 \pm 2.6 \ (45-51)$		$35 \pm 1.9 (33 - 37)$	
gubernaculum length	21 ± 1.6 (18−23)	ı	$17 \pm 0.5 (16-17)$	

**Type host and locality.** Isolated from a beetle of the genus *Mimela* (Coleoptera: Rutelidae) at a dumpster near Ganquan, Shaanxi province, PRC.

**Type material and type strain.** Type strain RS6138 frozen at the nematode collection of the MPI Tübingen and available as living culture upon request. Voucher specimens sent to the following museums: Holotype male, Para-type male and female: Museum für Naturkunde Karlsruhe, Germany; Paratype male and female: Swedish Natural History Museum, Stockholm, Sweden; Paratype male and female: University of California in Riverside Nematode Collection (UCRNC), Riverside, CA, USA.



**FIGURE 52.** The phylogeny represents a maximum likelihood tree that was computed from a concatenated alignment of more than 800 orthologous proteins with the LG substitution model. The stars denote branches with full support after 100 bootstrap pseudoreplicates.

# Discussion

This study describes nine novel *Pristionchus* species from Mainland China. As a result, the genus *Pristionchus* currently contains 48 species with whole transcriptome-based phylogenetic support and living cultures for active research. The fact that only two restricted sampling trips to two provinces in China resulted in an increase in the number of nominal species in the genus of roughly 20% strongly suggests that *Pristionchus* species sampling is far from saturation. Thus, additional collecting trips to China will likely add more interesting new species and life history traits to the *Pristionchus* clade. In contrast to previous sampling trips in Taiwan and Hong Kong, the species described in this study cover almost all phylogenetic clades within the genus, with the exception of the *pacificus*-group *senso stricto*.

In the present study, the phylogenetic relationship, *i.e.*, the topology of the phylogenetic tree is different from the previous study (Rödelsperger *et al.*, 2018). One particular subclade, previously part of the *pacificus*-group is transferred to the *maupasi*-group. This example indicates that additional samplings and the identification of new species can have an influence on topology and therefore, no given phylogenetic tree should be considered ultimate. Still, the phylogenetic groups are largely in accordance to their geographic distribution, *i.e.*, *maupasi* and *ento-mophagus*-groups are distributed mostly in Europe and North-America, respectively, whereas the other groups are mostly from Asia. However, the Asian *pacificus*-group *s.l.* is now split, and a subclade including *P. dorci* **n. sp.** and *P. purgamentorium* **n. sp.** collected from China, are closer to the American clade (*maupasi*-group *s.s.*) than to the *pacificus*-group *s.s.*. Thus, further sampling from these regions are necessary to provide additional information about their species diversification in relation to biogeography.

The species described in this study contain two unexpected but exciting findings. First, with *P. nudus* **n. sp.**, this study includes a novel species with unique morphological features. Specifically, the elongation of lateral sensilla, the apparent loss of cephalic papillae in males and the reduction of longitudinal striation resulting in an almost 'naked' appearance of the cuticle, indicate that novel morphological features can be identified in an already heavily sampled genus of nematodes. From a developmental perspective, this finding also indicates that conserved morphological features can be subject to evolutionary change in individual species or lineages. Thus, the phenotypic space of *Pristionchus* and other free-living nematodes is likely far from being fully explored. Second, *P. chinensis* **n. sp.** is the eighth hermaphroditic species in the genus *Pristionchus*. With one sixth (8/48) of *Pristionchus* species being androdioecious, the genus shows a remarkable plasticity in sex determination, unprecedented in any other nematode taxon. Taken together, China represents a potentially lucrative and promising region to search for new *Pristionchus* species and their associated life history traits, probably still harboring a plethora of yet undescribed nematodes that should be studied in more detail.

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