

Recharacterisation of three *Parasitodiplogaster* species based on morphological and molecular profiles

Natsumi KANZAKI^{1,2,*}, Robin M. GIBLIN-DAVIS¹, Weimin YE^{1,**},
Edward Allen HERRE³ and Barbara J. CENTER¹

¹ Fort Lauderdale Research and Education Center, University of Florida-IFAS,
3205 College Avenue, Davie, FL 33314-7799, USA

² Forest Pathology Laboratory, Forestry and Forest Products Research Institute,
1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan

³ Smithsonian Tropical Research Institute, Balboa, Ancon, Republic of Panama

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Summary – Three previously described *Parasitodiplogaster* spp., *P. nymphanema*, *P. obtusinema* and *P. trigonema* were re-isolated from their type locality, Barro Colorado Island Research Station, Smithsonian Tropical Research Institute, in Panama. The re-isolated materials were morphologically observed to compare with the original descriptions and molecularly characterised by DNA sequences of the near-full-length small subunit and D2-D3 expansion segments of the large subunit of the ribosomal RNA genes. Although the male tail characters, *i.e.*, arrangement of genital papillae and spicule and gubernaculum morphologies, were close to the original descriptions, a compressed stoma with two (right subventral and dorsal) teeth was observed for the first time and confirmed in the newly re-isolated materials. The molecular phylogenetic analysis revealed that the three re-isolated species are close to *P. laevigata*, *P. citrinema* and *P. popenema*, forming the *P. laevigata* group in the genus, and this group was separated into three subgroups, *P. citrinema* + *P. popenema* (subgroup 1), *P. nymphanema* + *P. obtusinema* (subgroup 2) and *P. laevigata* + *P. trigonema* and three undescribed species (subgroup 3). The *P. laevigata* group is characterised by a relatively compressed stoma with two (right subventral and dorsal) teeth, arrangement of genital papillae (three or four precloacal and four postcloacal pairs), but distinguishable from each other by stomatal morphology, *i.e.*, the shortest and most compressed being in subgroup 3, widest in subgroup 1, and intermediate in subgroup 2. Furthermore, a large and long spicule and gubernaculum were observed in subgroup 2 as its hypothesised apomorphy.

Keywords – description, *Ficus* spp., fig tree, genotyping, morphotyping, Panama, *Parasitodiplogaster nymphanema*, *Parasitodiplogaster obtusinema*, *Parasitodiplogaster trigonema*, taxonomy.

Nematodes associating with fig syconia show highly derived morphological and physiological characters compared with their phylogenetically close relatives (Giblin-Davis *et al.*, 2006; Davies *et al.*, 2008, 2015; Kanzaki *et al.*, 2009, 2014a; Susoy *et al.*, 2016). Thus, detailed morphological analyses of these nematodes in relation to their phylogenetic status are expected to provide important information about their morphological (structural) diversification and adaptation.

Parasitodiplogaster Poinar, 1979 is an associate of fig syconia (*Ficus* spp.) and fig wasps and has been reported from tropical and subtropical areas from all over the

world. It was first established as a monotypic genus with the description of the type species, *P. sycophilon* Poinar, 1979 isolated from *F. burkei* from Zimbabwe (Poinar, 1979), and subsequently followed by descriptions of ten species from ten different fig species from Barro Colorado Island, Panama (Smithsonian Tropical Research Institute); namely, *P. citrinema* Poinar & Herre, 1991 from *F. citrifolia*, *P. duganema* Poinar & Herre, 1991 from *F. dugandii*, *P. maxinema* Poinar & Herre, 1991 from *F. maxima*, *P. nymphanema* Poinar & Herre, 1991 from *F. nymphafolia*, *P. obtusinema* Poinar & Herre, 1991 from *P. obtusifolia*, *P. paranema* Poinar & Herre, 1991 from

* Corresponding author, e-mail: nkanzaki@affrc.go.jp

** Present address: Nematode Assay Section, Agronomic Division, North Carolina Department of Agriculture & Consumer Services, 4300 Reedy Creek Road, Raleigh, NC 27607, USA.

F. paraensis, *P. pertanema* Poinar & Herre, 1991 from *F. pertusa*, *P. popenema* Poinar & Herre, 1991 from *F. popenoei*, *P. trigonema* Poinar & Herre, 1991 from *F. trigonata*, and *P. yoponema* Poinar & Herre, 1991 from *F. yoponensis* (Poinar & Herre, 1991).

However, at the time all species were described based upon ethanol-fixed materials and thus several important morphological characters were potentially obfuscated during fixation. For example, the stoma of the species was described as being a blunt tube-like structure with no tooth or glottoid apparatus (Poinar & Herre, 1991).

Thereafter, Giblin-Davis *et al.* (2006) described *P. laevigata* Giblin-Davis, Ye, Kanzaki, Williams, Morris & Thomas, 2006 based upon live materials, and examined its ultrastructure and molecular phylogenetic status using scanning and transmission electron microscopy (SEM and TEM) and the D2-D3 expansion segments of the 28S ribosomal RNA gene (D2-D3 LSU). In that study, some morphological similarities between *P. laevigata* and the phylogenetically close genus *Allodiplogaster* Paramonov & Sobolev in Skrzjabin, Shikobalova, Sobolev, Paramonov & Sudarikov, 1954 were recognised, *e.g.*, the presence of dorsal and right subventral metastegostomatal teeth and telostegostomatal apodemes (Giblin-Davis *et al.*, 2006). Based on those findings, Kanzaki *et al.* (2010, 2013) and Wöhr *et al.* (2014) re-examined several *Parasitodiplogaster* species, and confirmed the presence of dorsal and right subventral teeth in *P. citrinema* and *P. popenema* (Kanzaki *et al.*, 2010), *P. maxinema* (Kanzaki *et al.*, 2013) and *P. sycophilum* (Wöhr *et al.*, 2014). Further, the presence of stomatal dimorphism in *P. maxinema* was reported, with a bacteria-feeding-like stenostomatous form and a predatory eurystomatous form, *i.e.*, some species of the genus may have saprophytic and predatory stages in addition to a potentially insect-parasitic life stage (Kanzaki *et al.*, 2013).

Currently, after including four additional species, *i.e.*, *P. australis* Bartholomaeus, Davies, Ye, Kanzaki & Giblin-Davis, 2009 from *F. virens* in Australia, *P. doliostoma* Kanzaki, Giblin-Davis, Davies & Center, 2012 from *F. sur* in Zimbabwe, *P. pharmaconema* Kanzaki, Giblin-Davis, Ye, Herre & Center, 2013 from *F. maxima* in Panama, and *P. salicifoliae* Wöhr, Greeff, Kanzaki & Giblin-Davis, 2015 from *F. ingens* and *F. salicifolia* in South Africa, the genus consists of 16 nominal species (Bartholomaeus *et al.*, 2009; Kanzaki *et al.*, 2012, 2013; Wöhr *et al.*, 2015).

In the present study, based upon newly re-isolated materials, three previously described *Parasitodiplogaster*

species, *P. trigonema*, *P. nymphanema* and *P. obtusinema*, were morphologically and molecularly recharacterised.

Materials and methods

NEMATODE ISOLATION, FIXATION AND MORPHOLOGICAL OBSERVATION

We surveyed Barro Colorado Island Research Station (BCI), Smithsonian Tropical Research Institute (STRI) in Panama (9°10'N latitude, 79°50'W longitude) in May 2008 and February 2010 for *Ficus* species (Harrison, 2005) that had been previously reported to harbour *Parasitodiplogaster* species (Poinar & Herre, 1991). Suitable phase B-D syconia were harvested and kept cool until dissected (within 3 days). Detailed procedures were described in previous studies (Kanzaki *et al.*, 2010, 2013).

The harvested figs were cut open with a scalpel and placed in distilled water for *ca* 5 min. Nematodes were handpicked alive into distilled water to rinse the surface and some of them stored in worm lysis solution for DNA extraction, amplification and sequencing attempts. Modified worm lysis buffer (Williams *et al.*, 1992; Ye *et al.*, 2007) and worm digestion solution (Tanaka *et al.*, 2012) were used for the 2008 and 2010 surveys, respectively. Otherwise, collected nematodes were heat-killed and placed into 5% formalin and then processed into 100% glycerin for permanent mounts (Southey, 1970). Some of the extra specimens were fixed into DESS (Yoder *et al.*, 2006) for back-up. The present study focused on *P. obtusinema* collected from *F. obtusifolia* in the 2008 survey, and *P. trigonema* from *F. trigonata* and *P. nymphanema* from *F. nymphaeifolia* collected in the 2010 survey.

MOLECULAR SEQUENCING AND PHYLOGENETIC ANALYSIS

The nematode materials stored in the lysis solution were digested according to the methods in Ye *et al.* (2007) and Tanaka *et al.* (2012), and the lysates were used as the PCR template DNA. The D2-D3 LSU and near-full-length of the small subunit of ribosomal RNA gene (SSU) of *Parasitodiplogaster* spp. were determined as described by Ye *et al.* (2007) and Kanzaki & Futai (2002), and compared with those stored in the GenBank database. The molecular sequences determined in the present study were deposited in the GenBank database with accession numbers LC109317-LC109320.

Table 1. GenBank accession numbers of the nematodes phylogenetically examined in the present study.

Genus	Species	SSU	D2D3 LSU	Note
<i>Koerneria</i>	cf. <i>luziae</i>	AB597232	AB597243	Outgroup for the family
<i>Allodiplogaster</i>	<i>josephi</i>	AB440306	AB440321	Outgroup for <i>Parasitodiplogaster</i> - <i>Teratodiplogaster</i> clade
<i>Teratodiplogaster</i>	<i>fignewmani</i>	AB440310	AB440312	Tentative sister genus of <i>Parasitodiplogaster</i>
<i>Teratodiplogaster</i>	<i>variegatae</i>	LC004467	LC004468	Tentative sister genus of <i>Parasitodiplogaster</i>
<i>Parasitodiplogaster</i>	<i>australis</i>	NA*	EU018051	<i>australis</i> group
<i>Parasitodiplogaster</i>	<i>salicifoliae</i>	NA*	KP015026	<i>australis</i> group
<i>Parasitodiplogaster</i>	<i>sycophilon</i>	NA*	KF211402	Not assigned in any groups so far
<i>Parasitodiplogaster</i>	sp. L1	NA*	AB901280	<i>maxinema</i> group
<i>Parasitodiplogaster</i>	sp. L2	NA*	AB901281	<i>maxinema</i> group
<i>Parasitodiplogaster</i>	sp. L3	NA*	AB901282	<i>maxinema</i> group
<i>Parasitodiplogaster</i>	sp. S1	AB901286	NA*	<i>maxinema</i> group
<i>Parasitodiplogaster</i>	sp. S2	AB901287	NA*	<i>maxinema</i> group
<i>Parasitodiplogaster</i>	sp. S3	AB901288	NA*	<i>maxinema</i> group
<i>Parasitodiplogaster</i>	sp. S4	AB901289	NA*	<i>maxinema</i> group
<i>Parasitodiplogaster</i>	<i>maxinema</i>	AB901283	AB901278	<i>maxinema</i> group
<i>Parasitodiplogaster</i>	<i>pharmaconema</i>	AB901284	AB810254	<i>maxinema</i> group
<i>Parasitodiplogaster</i>	<i>popenema</i>	NA*	AY840560	<i>laevigata</i> group 1
<i>Parasitodiplogaster</i>	<i>citrinema</i>	AB901285	AY840555	<i>laevigata</i> group 1
<i>Parasitodiplogaster</i>	<i>trigonema</i>	NA*	AY840562	<i>laevigata</i> group 3
<i>Parasitodiplogaster</i>	<i>laevigata</i>	NA*	AY840558	<i>laevigata</i> group 3
<i>Parasitodiplogaster</i>	sp. RGD239	NA*	AY840561	<i>laevigata</i> group 3
<i>Parasitodiplogaster</i>	sp. RGD579	AB440307	EU018054	<i>laevigata</i> group 3
<i>Parasitodiplogaster</i>	sp. RGD580	AB501144	AB501143	<i>laevigata</i> group 3
<i>Parasitodiplogaster</i>	<i>nymphanema</i>	LC109317	LC109318	<i>laevigata</i> group 2
<i>Parasitodiplogaster</i>	<i>obtusinema</i>	LC109319	LC109320	<i>laevigata</i> group 2

* Not available because the sequence was not deposited in the database or not successfully sequenced by authors.

The molecular phylogenetic relationship was inferred based on the SSU and D2-D3 LSU. The sequences obtained were compared with those of other diplogastrid species belonging to the *Allodiplogaster* + *Teratodiplogaster* Kanzaki, Giblin-Davis, Davies, Ye, Center & Thomas, 2009 + *Parasitodiplogaster* clade and outgroup taxa, *Koerneria* cf. *luziae* (Körner, 1954) Meyl, 1960 deposited in the GenBank database. The GenBank accession numbers of the compared sequences are summarised in Table 1.

The molecular phylogenetic analyses were conducted using Bayesian analyses. The compared sequences were aligned separately using MAFFT (Katoh *et al.*, 2002; available online at <http://mafft.cbrc.jp/alignment/server/index.html>) and the base substitution model was determined for each gene locus using MODELTEST version 3.7 (Posada & Crandall, 1998) under the AIC model selection criterion, and GTR + I + G and TrN + G were selected for SSU and D2-D3 LSU, respectively. The Akaike-supported model, log likelihood (ln L), Akaike in-

formation criterion values, proportion of invariable sites, gamma distribution shape parameters and substitution rates were applied to the phylogenetic analysis. Bayesian analysis was performed using MrBayes 3.2 (Huelsenbeck & Ronquist, 2001); four chains were run for 4×10^6 generations. Markov chains were sampled at intervals of 100 generations (Larget & Simon, 1999). Two independent runs were performed and after confirming the convergence of runs and discarding the first 2×10^6 generations as burn-in, the remaining topologies were used to generate a 50% majority-rule consensus tree.

Results

MORPHOLOGICAL OBSERVATION

The species identities were confirmed based on general morphology and morphometric values described in the original description (Poinar & Herre, 1991). The newly re-isolated specimens were generally identical to their

respective original descriptions except for several key morphological characters, specifically stomatal morphology, and morphometric values that can be highly variable within a species.

The general morphology of these three species is similar except for some stomatal and male tail characters. Thus, common morphological traits are described first and the characters of each species are then described with reference to the original descriptions.

COMMON MORPHOLOGICAL CHARACTERS OF
PARASITODIPLOGASTER TRIGONEMA, *P. NYMPHANEMA*
AND *P. OBTUSINEMA*

Adults

Large species. Body length varying from 1.5-3.0 mm, sometimes in excess of 3.0 mm, cuticle with coarse and shallow annulation, without clear longitudinal striation. In fixed and mounted specimens, a lateral field-like structure can be observed, but not clearly distinguished as to whether it is a real lateral field or a fold occurring during fixation and dehydration. Deirid difficult to observe, but sometimes found at level just posterior to basal bulb. Lip region not clearly separated from body, *i.e.*, no clear constriction observed, weakly separated into six equal-sized sectors. Each lip sector with small labial sensilla, but not clearly observed in most individuals. Both males and females with a cephalic dome (= cephalic sensillum) on subventral and dorso-lateral sectors. Stoma narrow, separated into three sections, cheilostom, gymnostom and stegostom. Cheilostom degenerate, forming a lightly cuticularised ring surrounding gymnostom at level of stomatal opening, difficult to observe. Gymnostom forming short cuticular tube, surrounded by cheilostom, subdivisions and arcade syncytia associated with gymnostom not distinguishable by light microscopy. Stegostom separated into three subsections, pro-mesostegostom, metastegostom and telostegostom. Pro-mesostegostom not clearly cuticularised, surrounding metastegostomatal teeth. Metastegostom forming claw-like dorsal and right subventral teeth. No clear cuticular armature observed on left subventral sector of metastegostom. Telostegostom forming cuticularised funnel connecting stoma and pharynx. Detailed structure of metastegostomatal teeth described for each species below. Anterior pharynx (procorpus + median bulb) long, weakly muscular; procorpus cylindrical, with grinder-like apparatus on its inner wall giving appearance of a cuticular lining in light microscopic observation, median bulb slightly elongate, long oval or rounded squared in lateral

view. Because median bulb is not well developed, margin between metacarpus and isthmus is difficult to distinguish in many individuals. Posterior pharynx (isthmus + terminal bulb) glandular, shorter than anterior pharynx, isthmus and basal bulb not distinctive. Cardia small, closed, not well developed. Hemizonid not observed. Nerve ring surrounding middle part of posterior pharynx. Excretory pore opening at level of, or anterior to basal bulb. Excretory duct surrounded by thick wall, very distinctive.

Male

Body ventrally arcuate when heat-killed. Tail region ventrally curved. Testis usually outstretched, on ventral side of body, usually on right of intestine. Spermatocytes arranged in multiple (2-3) rows at 1/5-1/6 gonad length from anterior part, and then well developed spermatocytes arranged in single row. *Vas deferens* not clearly distinguished from testis. Tail characters are described for each species below.

Female

Body weakly curved dorsally when heat-killed. Ovaries didelphic and antidromously reflexed (= each ovary reflexed for its entire length). Anterior reproductive tract situated on right of intestine, posterior tract on left. Anterior and posterior reproductive tracts almost identical to each other so only anterior one described here. Ovary extended, sometimes reaching vulval region, oocytes arranged in multiple rows in distal part, in single file in posterior part. Oviduct serving as uterus and spermatheca, containing two to more than 40 eggs at single- or two-celled stage in mature female. Vulva protuberant, four large vaginal gland cells present. Vagina perpendicular to body surface. Vulval muscle conspicuous, filaments arranged as quarter sector in lateral view. Rectum *ca* one anal body diam. long. Intestine-rectum junction constricted by sphincter muscle. Tail weakly tapering, ending in short, cone-like, or rounded tip, a small mucron sometimes present. Phasmid pore-like, near tail tip.

SPECIES-SPECIFIC MORPHOLOGICAL CHARACTERS

***Parasitodiplogaster trigonema* Poinar & Herre, 1991**
(Figs 1-4; Table 2)

Lip and stomatal morphology

Anterior end appearing somewhat square in lateral view, although this could be an artefact as teeth of all ex-

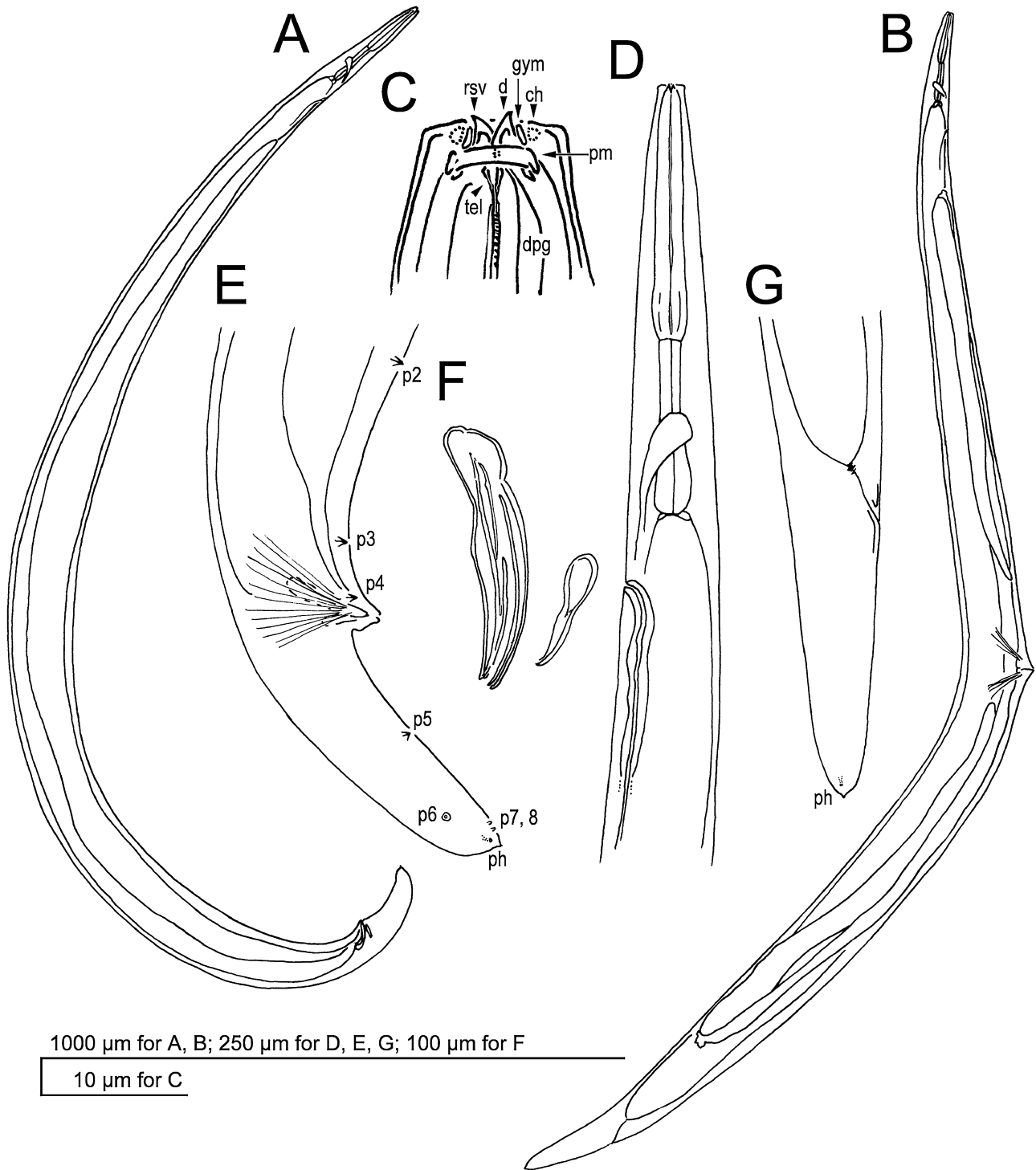


Fig. 1. *Parasitodiplogaster trigonema*. A: Male; B: Female; C: Left lateral view of stomatal region; D: Neck region; E: Male tail showing second to eighth pairs of genital papillae; F: Spicule and gubernaculum; G: Female tail. Abbreviations: right subventral tooth (rsv), dorsal tooth (d), cheilostom (ch), gymnostom (gym), pro-mesostegostom (pm), telostegostom (tel), dorsal pharyngeal gland (dpg), genital papillae from anterior where first pair is far anterior to cloacal opening (p + number) and phasmid (ph).

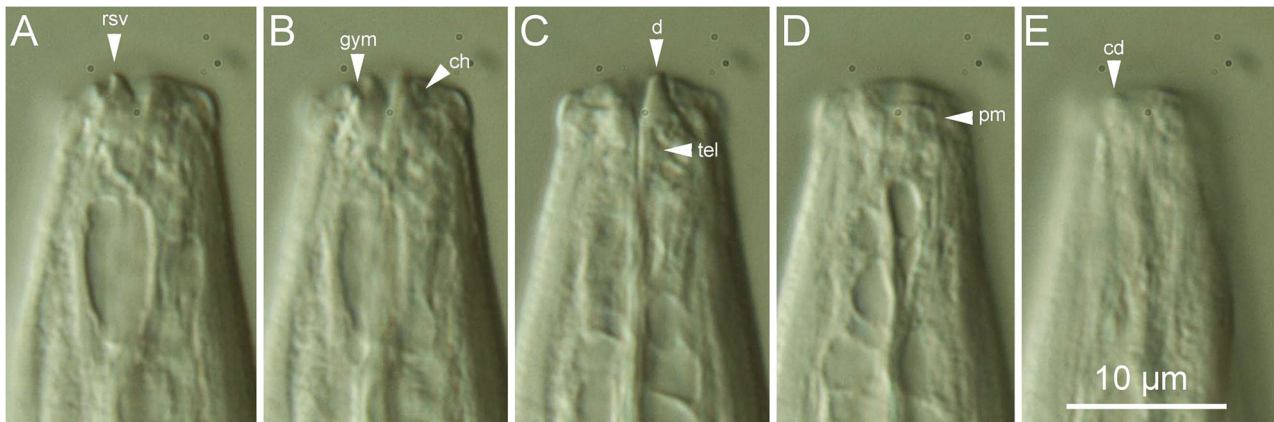


Fig. 2. Stomatal region of *Parasitodiplogaster trigonema* in different focal planes. Abbreviations: right subventral tooth (rsv), dorsal tooth (d), cheilostom (ch), gymnostom (gym), pro-mesostegostom (pm), telostegostom (tel) and cephalic dome (cd). This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/15685411>.

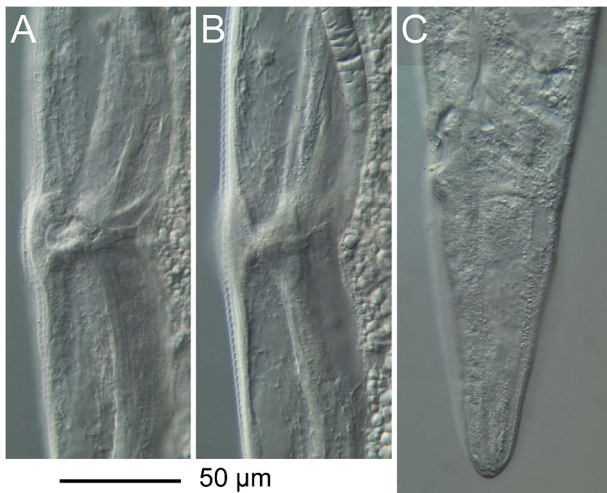


Fig. 3. Adult female of *Parasitodiplogaster trigonema*. A, B: Left subventral view of vulval region in different focal planes; C: Tail region. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/15685411>.

amined individuals were protracted, possibly during fixation, causing anterior end to expand. Two short and stout metastegostomatal teeth, forming a somewhat triangular shape. Two teeth close to each other, appearing as partially overlapping and obscuring stomatal opening in lateral view.

Male tail character

Spicules separate, wide, stout; manubrium rounded with small notch or depression at middle of anterior end;

blade appearing ventrally curved with small expansion on ventral side at one-third of distance from anterior end and bluntly rounded distal end. Gubernaculum in lateral view slender, anterior end rounded, posterior half ventrally curved with bluntly pointed distal end. Tail abruptly narrowing just posterior to cloacal opening, broad, ventrally arcuate, slightly tapering to distal end, distal end forming short cone, sometimes with short projection (mucron). Eight pairs of subventral genital papillae present: first pair, *ca* 4-5 cloacal body diam. (CBD) anterior to cloacal opening (CO), second pair *ca* two CBD anterior to CO, third pair at less than one CBD anterior to CO, fourth pair just anterior to CO, fifth pair *ca* one CBD posterior to CO, sixth lateral pair three-quarters of way from fifth pair and tail tip, seventh and eighth pairs close to each other, just anterior to tail tip, phasmid laterally opening almost at same level or slightly posterior to seventh pair.

COMPARISON WITH ORIGINAL DESCRIPTION

Compared with the original description, several minor and major differences were confirmed. Both anterior and posterior pharynx were described as glandular in the original description (Poinar & Herre, 1991), but the structure was similar to recently described or redescribed species, *i.e.*, the anterior pharynx is weakly muscular and the posterior pharynx is glandular (Giblin-Davis *et al.*, 2006; Bartholomaeus *et al.*, 2009; Kanzaki *et al.*, 2010, 2012, 2013, 2014b; Wöhr *et al.*, 2014, 2015).

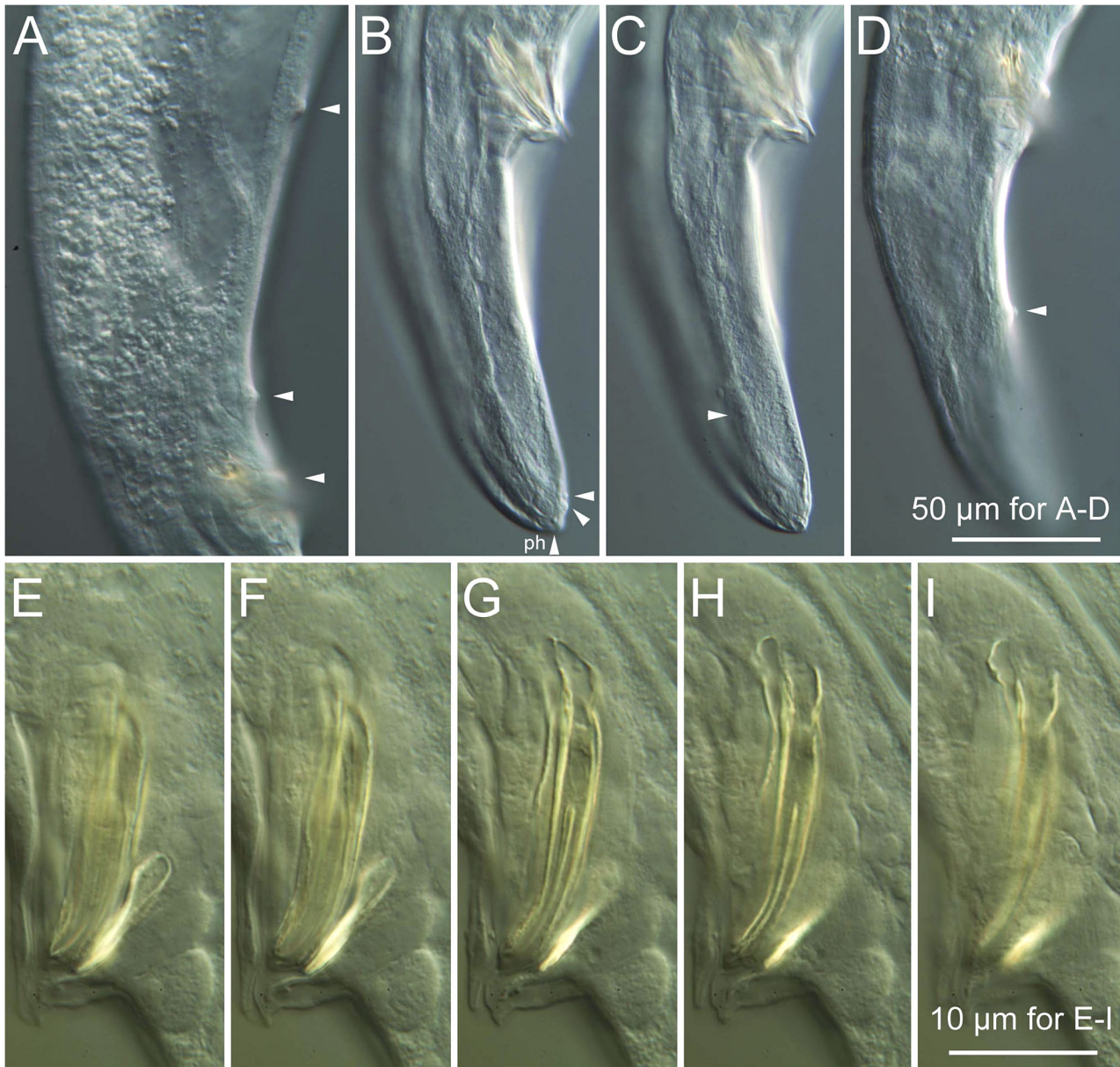


Fig. 4. Adult male of *Parasitodiplogaster trigonema*. A: Anterior part of tail showing second to fourth pairs of genital papillae with arrowheads (first pair is far anterior to cloacal opening); B-D: Posterior part of tail in different focal planes showing fifth to eighth pairs of genital papillae (arrowheads) and phasmid (ph); E-I: Close-up of spicule and gubernaculum in different focal planes. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/15685411>.

The detailed stomatal structure was not given in the original description. The stoma was only described as “small” (Poinar & Herre, 1991), and the detailed lip and stomatal structures were not described. However, this

species has weakly separated lips with labial papillae and cephalic domes (= homologous to cephalic sensilla) and a relatively specialised and complex stoma as described above.

Table 2. Morphometric values of *Parasitodiplogaster trigonema*. All measurements are in μm . Values obtained in the present study are in the form: mean \pm s.d. (range), and those from the original description: mean (range).

Character	Male		Female	
	Present study	Original description	Present study	Original description
n	10	10	10	10
L	2381 \pm 378 (1797-2841)	2528 (2020-3040)	2614 \pm 308 (2285-3205)	2556 (2070-3330)
a	22.1 \pm 2.1 (19.7-26.9)	Not given	22.2 \pm 2.9 (17.0-25.1)	Not given
b	13.4 \pm 1.4 (11.6-15.3)	Not given	14.1 \pm 1.1 (12.4-15.9)	Not given
c	17.6 \pm 1.9 (15.3-21.1)	Not given	16.9 \pm 1.6 (15.2-20.3)	Not given
c'	2.6 \pm 0.3 (2.3-3.4)	Not given	2.9 \pm 0.5 (2.1-3.6)	Not given
T or V	80.2 \pm 3.3 (74.9-86.4)	Not given	50.9 \pm 1.7 (48.5-54.1)	51 (48-55)
Anterior pharynx length	104 \pm 9.6 (90-117)	108 (88-123)	109 \pm 5.7 (97-116)	99 (66-120)
Posterior pharynx length	69 \pm 9.0 (58-90)	75 (63-92)	71 \pm 6.7 (58-80)	74 (60-90)
Anterior/posterior pharynx ratio	1.52 \pm 0.1 (1.31-1.73)	1.42 (1.27-1.75)	1.54 \pm 0.1 (1.43-1.68)	1.37 (1.14-1.54)
Nerve ring from anterior end	141 \pm 13 (128-168)	143 (110-181)	145 \pm 9.0 (126-156)	128 (90-159)
Excretory pore from anterior end	212 \pm 24 (172-254)	218 (187-247)	238 \pm 20 (207-275)	210 (171-279)
Excretory pore from basal bulb	35 \pm 14 (13-61)	Not given	52 \pm 13 (36-83)	Not given
Max. body diam.	108 \pm 16 (83-138)	99 (79-130)	118 \pm 12 (103-135)	129 (99-156)
Vulval body diam.	–	–	114 \pm 13 (96-135)	Not given
Testis length	1911 \pm 315 (1402-2317)	Not given	–	–
Anterior ovary length	–	–	773 \pm 70 (649-860)	Not given
Posterior ovary length	–	–	771 \pm 110 (608-924)	Not given
Cloacal or anal body diam.	53 \pm 6.5 (45-65)	58 (48-64)	55 \pm 7.7 (41-67)	52 (42-63)
Tail length	135 \pm 18 (109-164)	135 (115-152)	156 \pm 21 (128-183)	162 (129-180)
Spicule length ¹	47 \pm 4.1 (42-55)	48 (36-54)	–	–
Gubernaculum length	27 \pm 3.2 (23-32)	24 (21-32)	–	–

¹ Spicule is relatively straight, and curved length and chord from anterior end of manubrium and distal end are not different.

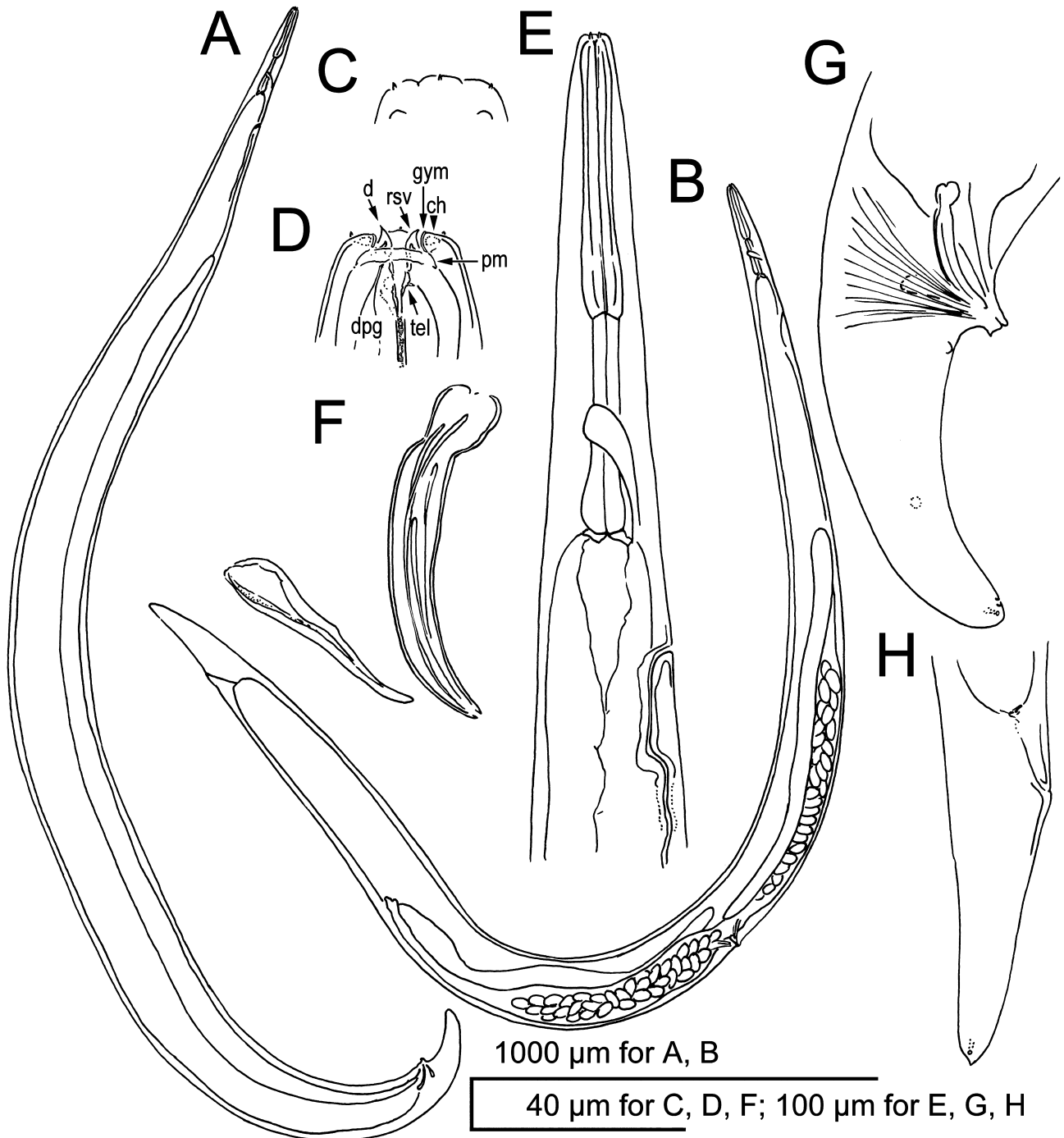


Fig. 5. *Parasitodiplogaster obtusinema*. A: Male; B: Female; C: Surface of lip region; D: Stomatal region; E: Neck region; F: Spicule and gubernaculum; G: Male tail (genital papillae were not observed clearly); H: Female tail. Abbreviations: right subventral tooth (rsv), dorsal tooth (d), cheilostom (ch), gymnostom (gym), pro-mesostegostom (pm), telostegostom (tel), dorsal pharyngeal gland (dpg).



Fig. 6. Stomatal region of *Parasitodiplogaster obtusinema* in different focal planes. Abbreviations: right subventral tooth (rsv), dorsal tooth (d), cheilostom (ch), gymnostom (gym), pro-mesostegostom (pm), telostegostom (tel), labial sensilla (ls) and cephalic dome (cd). This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/15685411>.

***Parasitodiplogaster obtusinema* Poinar & Herre, 1991**
(Figs 5-8; Table 3)

Lip and stomatal morphology

Anterior end appearing somewhat square in lateral view. However, teeth of all examined individuals were protracted which might have been due to a fixation artefact resulting in an expansion of anterior end. Both metastegostomatal teeth stout, each with a somewhat rose-thorn-like shape in lateral view.

Male tail characters

Spicules separate, stout, manubrium squared with small notch or depression at middle of anterior end, most of blade weakly ventrally curved with curvature becoming stronger at its distal part. Gubernaculum in lateral view slender, anterior end rounded; posterior half ventrally curved with bluntly pointed distal end. Tail broad, ventrally arcuate, slightly tapering to distal end, distal end forming short cone, sometimes with short projection (mucron). Genital papillae not clearly observed, probably because of specimen condition. Only four pairs of papillae: a subventral pair slightly posterior to CO, a lateral pair near mid-tail and two small subventral pairs just anterior to tail tip, were observed in some individuals. Phasmid opening

laterally at almost same level or slightly posterior to most distal genital papilla.

COMPARISON WITH ORIGINAL DESCRIPTION

Both anterior and posterior pharynx were described as muscular with valve apparatus in the original description (Poinar & Herre, 1991), but the structure was similar to other recently described or redescribed species, *i.e.*, the anterior pharynx is weakly muscular and the posterior pharynx is glandular (see above).

The detailed stomatal structure was not given in the original description. The stoma was described as “small” (Poinar & Herre, 1991) and the detailed lip and stomatal structures were not described. However, this species has weakly separated lips with labial papillae and cephalic domes and a relatively complex stoma as described above.

Seven (three precloacal and four postcloacal) pairs of genital papillae were reported in the original description. The three (first, third and fourth) pairs found in the present study correspond with the fourth postcloacal pair and two terminal ventral pairs. However, the second pair does not correspond to any of the originally described pairs and could be an artefact. The arrangement of genital papillae needs further confirmation using freshly-isolated specimens.

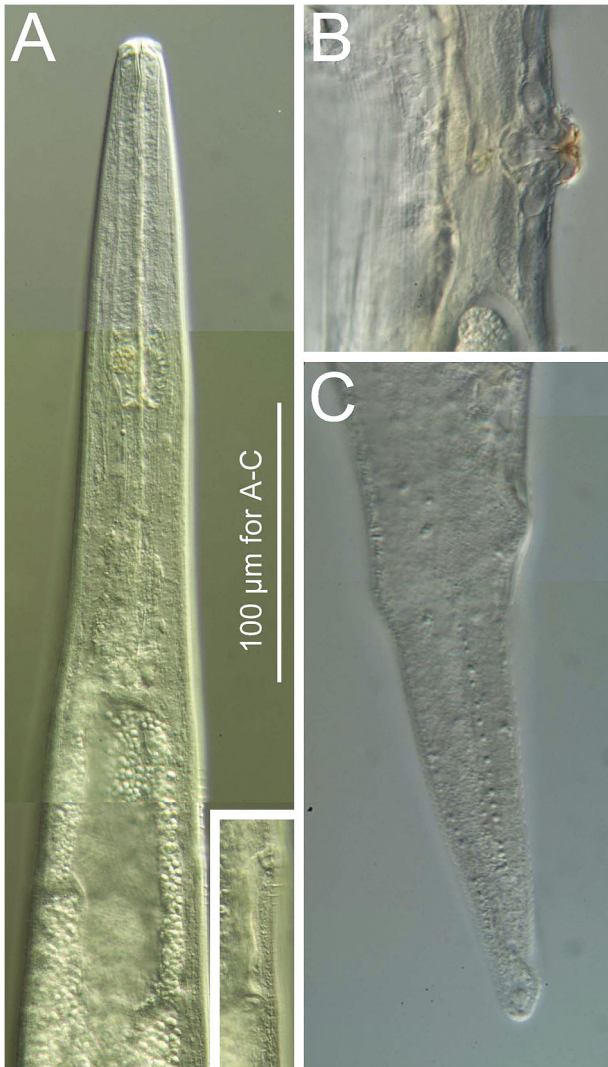


Fig. 7. Adult female of *Parasitodiplogaster obtusinema*. A: Neck region showing excretory-secretory system (enclosed in rectangle); B: Vulval opening region; C: Tail. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/15685411>.

***Parasitodiplogaster nymphanema* Poinar & Herre, 1991**
(Figs 9-12; Table 4)

Lip and stomatal morphology

Anterior end appearing somewhat square in lateral view, but this could be an artefact, teeth of all examined individuals being protracted, possibly as a result of fixation, and which might have expanded anterior end.

Two short and stout metastegostomatal teeth, each with a somewhat triangular shape in lateral view.

Male tail characters

Spicules separate, with complex structure, manubrium large, forming a somewhat ear-like shape, *i.e.*, roundish rectangle with smooth depression on dorsal side, blade ventrally bent at just posterior to manubrium, otherwise overall shape appearing straight. Clear and smooth dorsal and ventral expansions present around middle part of spicule blade, blade then smoothly narrowing to a pointed and weakly dorsally recurved distal end. Gubernaculum in lateral view slender, anterior end bluntly pointed and ventrally curved, slightly dorsally curved at mid-point, distal tip bluntly pointed and ventrally curved. Tail abruptly narrowing just posterior to cloacal opening, broad, ventrally arcuate, slightly tapering to distal end, distal end forming short cone, sometimes with short projection (mucron). Eight pairs of genital papillae present: first pair, *ca* 4-5 CBD anterior to CO; second pair *ca* 2.5 CBD anterior to CO, third pair at 1.5 CBD anterior to CO, fourth pair <1 CBD anterior to CO; fifth pair *ca* half CBD posterior to CO, sixth lateral pair close to fifth pair, one-fifth way from fifth pair and tail tip, seventh and eighth pairs close to each other, just anterior to tail tip, phasmid located laterally near tail tip, mid-way between eighth pair and tail tip.

COMPARISON WITH ORIGINAL DESCRIPTION

Both anterior and posterior pharynx were described as “well-developed and muscular” in the original description (Poinar & Herre, 1991), but the structure was similar to recently described or redescribed species, *i.e.*, the anterior pharynx is weakly muscular and the posterior pharynx is glandular (see above).

The stoma was originally described only as “distinct, longer than wide” (Poinar & Herre, 1991), and the lip structures were not described. However, the species has weakly separated lips with labial papillae and cephalic domes and a relatively complex stoma as detailed above.

In the original description, seven pairs of genital papillae were reported, the distal (seventh) pair being described thus: “terminal tail papilla pair have two nerve endings each”. This pair corresponds to the seventh and eighth terminal pairs found in the present study, and each nerve ending corresponds to a single papilla. Otherwise, the arrangement found in the present study matches the original description.

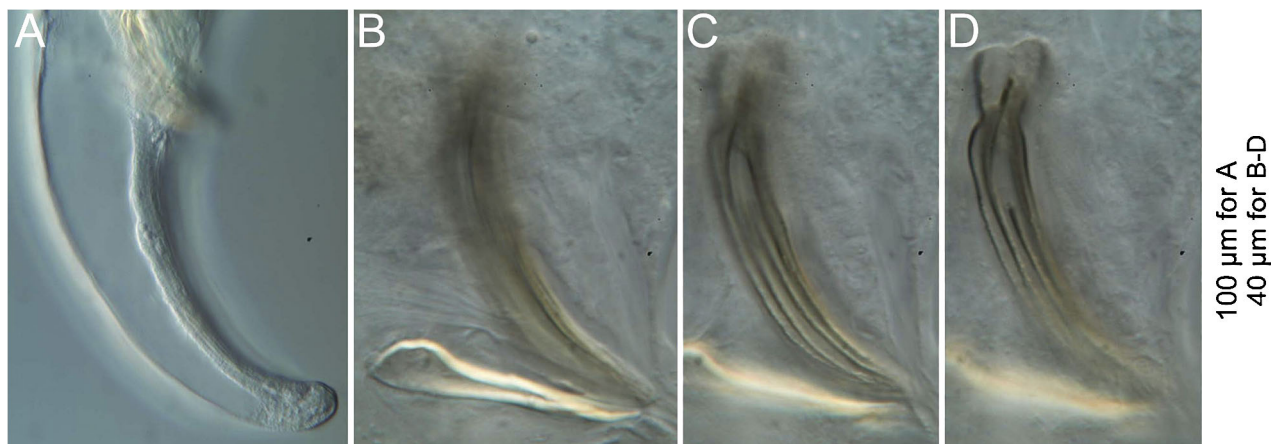


Fig. 8. Adult male of *Parasitodiplogaster obtusinema*. A: Tail region; B-D: Close-up of spicule and gubernaculum in different focal planes. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/15685411>.

MOLECULAR PHYLOGENETIC RELATIONSHIPS

The genus *Parasitodiplogaster* was separated into four phylogenetic groups (Fig. 13) as suggested previously (Kanzaki *et al.*, 2014b; Wöhr *et al.*, 2014, 2015). The *P. australis* group is clearly separated from the other groups with the rest being separated into three clades: the *P. sycophilon* group, the *P. laevigata* group and the *P. maxinema* group. Further, the *P. laevigata* group is separated into three subclades: *P. citrinema* + *P. popenema* (subgroup 1), *P. nymphanema* + *P. obtusinema* (subgroup 2) and *P. laevigata* + *P. trigonema* + three undescribed species (subgroup 3) (Fig. 13).

Discussion

Detailed comparisons between morphological characters and phylogenetic relationships have been previously conducted for *Parasitodiplogaster* spp. (Giblin-Davis *et al.*, 2006; Kanzaki *et al.*, 2010, 2012, 2014b; Wöhr *et al.*, 2014, 2015). Therefore, several characters confirmed in the present study are briefly discussed here.

Three species examined in the present study were separated into two subgroups, *i.e.*, *P. nymphanema* and *P. obtusinema* formed an independent subgroup 2, and *P. trigonema* was close to *P. laevigata* and three other undescribed species from Central America (RGD239, RGD579 and RGD580) forming subgroup 3. After including our unpublished observations on these undescribed species, these subclades, which have all been isolated from the North American Meridian from subgenus

Urostigma, section *Americana* figs can be characterised by their stomatal morphology and the patterns of their genital papillae arrangement.

The members of subgroups 2 and 3 have a degenerate cheilostom, short tube-like gymnostom and stegostom with very stout claw-like or triangular and singular right subventral and dorsal teeth (= metastegostomatal teeth). Furthermore, as a generic character, the left subventral metastegostomatal sector is bereft of any of the teeth, ridges or denticles that are usually found in other diplogastrids (see Sudhaus & Fürst von Lieven, 2003; Susoy *et al.*, 2015).

Based on the stomatal morphology, the subgroups are distinguished from each other, and from the other groups, *i.e.*, subgroup 1 (*P. citrinema* and *P. popenema*) has a wide stoma with diamond-shaped and singular right subventral and dorsal teeth (Kanzaki *et al.*, 2010), subgroup 3 has a compressed stoma where two teeth occupy the stomatal opening, and subgroup 2 is somewhat intermediate between subgroups 1 and 3. The *P. australis* group has a tube-like stoma lacking teeth or denticles, but possesses two squared cheilostomatal projections at the stomatal opening (Bartholomaeus *et al.*, 2009; Wöhr *et al.*, 2015), whilst the *P. maxinema* group has a long and tube-like stoma with stick-like and single right subventral and dorsal teeth (Kanzaki *et al.*, 2013, 2014b). The stomatal structure of the *P. laevigata* group is similar to the *P. sycophilon* group forming an independent clade neighbouring the *P. laevigata* group and the *P. maxinema* group, *i.e.*, they share a degenerate cheilostom, short-tube-like gymnostom and short and stout and singular

Table 3. Morphometric values of *Parasitodiplogaster obtusinema*. All measurements are in μm . Values obtained in the present study are in the form: mean \pm s.d. (range), and those from the original description: mean (range).

Character	Male		Female	
	Present study	Original description	Present study	Original description
n	10	8	10	2
L	2893 \pm 673 (1986-3594)	2670 (1920-3990)	3212 \pm 658 (2348-4159)	2550, 2940
a	24.1 \pm 5.0 (14.3-29.6)	Not given	19.9 \pm 1.5 (18.4-23.2)	Not given
b	13.7 \pm 2.6 (9.0-16.8)	Not given	15.6 \pm 1.9 (13.0-18.5)	Not given
c	19.0 \pm 3.5 (11.9-23.7)	Not given	21.6 \pm 2.6 (17.8-26.0)	Not given
c'	2.2 \pm 0.2 (2.0-2.6)	Not given	3.0 \pm 0.4 (2.1-3.6)	Not given
T or V	73.2 \pm 7.0 (54.8-80.8)	Not given	50.3 \pm 1.8 (47.8-53.7)	50, 52
Anterior pharynx length	112 \pm 11 (96-128)	106 (93-120)	110 \pm 13 (91-128)	99, 105
Posterior pharynx length	91 \pm 6.9 (81-101)	85 (66-99)	90 \pm 11 (65-106)	78, 81
Anterior/posterior pharynx ratio	1.23 \pm 0.1 (1.12-1.39)	1.25 (1.11-1.54)	1.23 \pm 0.1 (1.11-1.40)	1.22, 1.35
Nerve ring from anterior end	174 \pm 14 (152-191)	150 (135-186)	166 \pm 23 (130-197)	141, 159
Excretory pore from anterior end	261 \pm 35 (210-297)	250 (174-315)	267 \pm 55 (194-333)	237, 276
Excretory pore from basal bulb	52 \pm 26 (10-101)	Not given	63 \pm 38 (13-114)	Not given
Max. body diam.	126 \pm 42 (71-197)	109 (84-174)	162 \pm 33 (107-206)	105-162
Vulval body diam.	–	–	153 \pm 27 (107-194)	Not given
Testis length	2139 \pm 590 (1088-2716)	Not given	–	–
Anterior ovary length	–	–	874 \pm 202 (661-1199)	Not given
Posterior ovary length	–	–	833 \pm 271 (573-1316)	Not given
Cloacal or anal body diam.	69 \pm 9.0 (54-87)	61 (53-75)	49 \pm 6.5 (43-62)	45, 51
Tail length	152 \pm 20 (125-181)	117 (90-153)	150 \pm 29 (96-184)	168, 168
Spicule length ¹	59 \pm 5.6 (51-68)	55 (48-68)	–	–
Spicule length ²	66 \pm 6.8 (55-74)	Not given	–	–
Gubernaculum length	39 \pm 4.8 (32-46)	31 (24-36)	–	–

¹ Spicule length in chord from anterior end of manubrium and distal end.

² Spicule length curved along middle part from anterior end of manubrium to distal end.

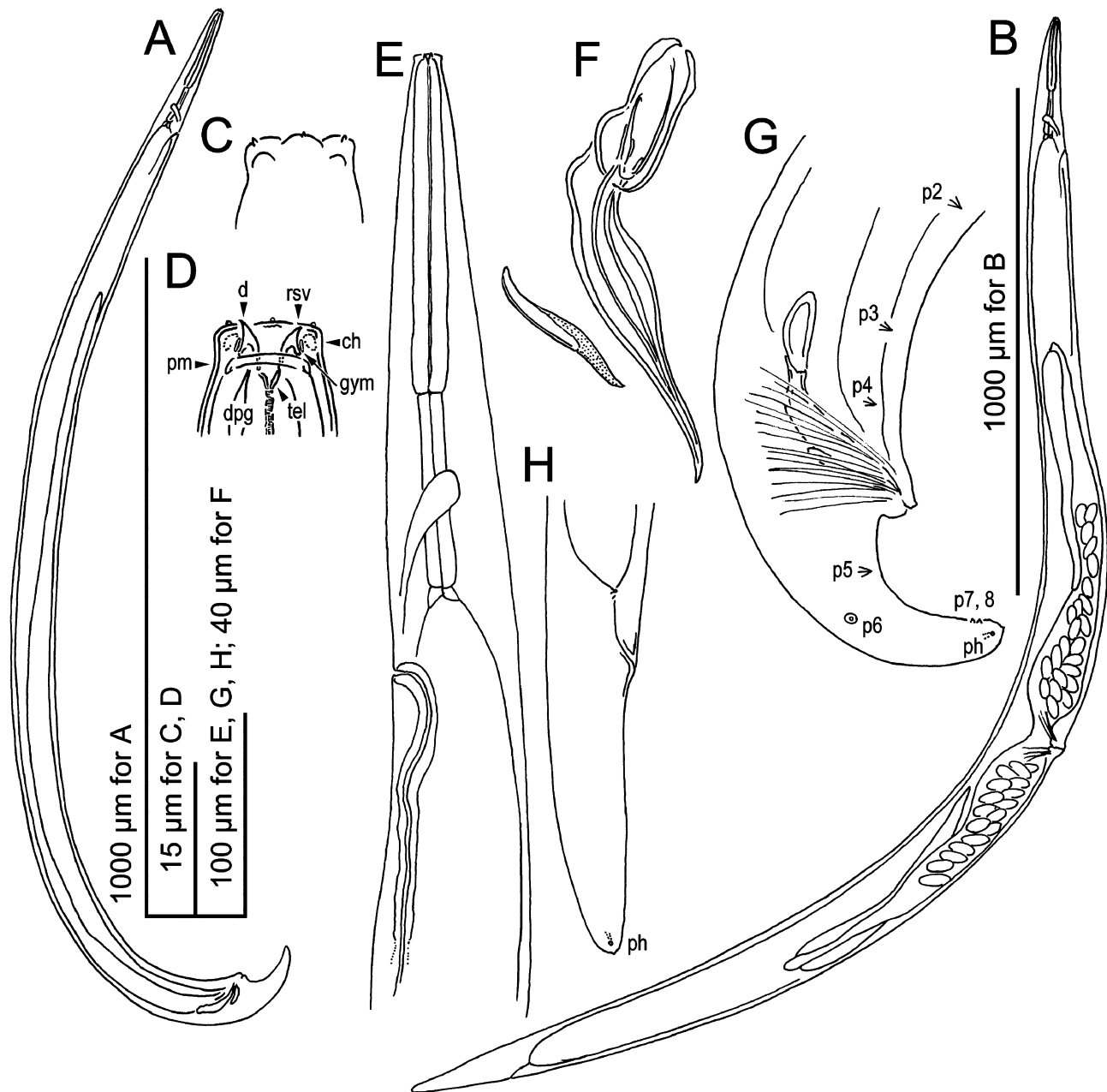


Fig. 9. *Parasitodiplogaster nymphanema*. A: Male; B: Female; C: Surface of lip region; D: Stomatal region; E: Neck region; F: Spicule and gubernaculum; G: Male tail showing second to fourth pairs of genital papillae where first pair is far anterior to cloacal opening and phasmid; H: Female tail. Abbreviations: right subventral tooth (rsv), dorsal tooth (d), cheilostom (ch), gymnostom (gym), promesostegostom (pm), telostegostom (tel), dorsal pharyngeal gland (dpg), genital papillae from anterior where first pair is far anterior to cloacal opening (p + number) and phasmid (ph).

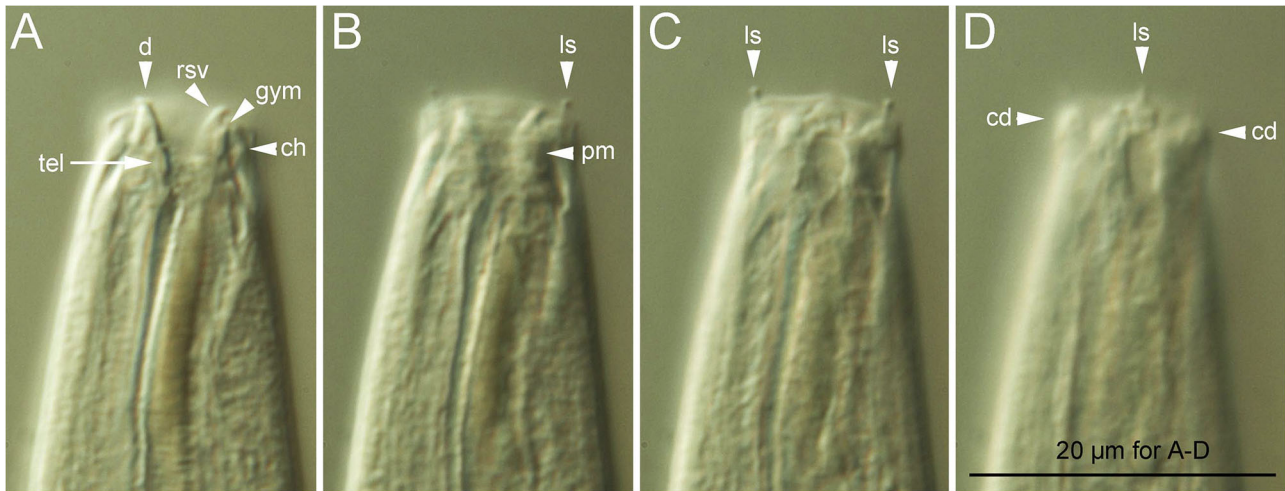


Fig. 10. Stomatal region of *Parasitodiplogaster nymphanema* in different focal planes. Abbreviations: right subventral tooth (rsv), dorsal tooth (d), cheilostom (ch), gymnostom (gym), pro-mesostegostom (pm), telostegostom (tel), labial sensilla (ls) and cephalic dome (cd). This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/15685411>.

dorsal and right subventral teeth (Wöhr *et al.*, 2014). This condition could represent the plesiomorphic state present in the common ancestor of those three clades, with the long stoma and stomatal dimorphism in the *P. maxinema* group representing an apomorphy (or reversion to an even earlier state).

The arrangement of genital papillae is also similar within each subgroup, with subgroups 2 and 3 being similar to each other. The members of subgroups 2 and 3 have three or four precloacal subventral pairs and four postcloacal pairs. The arrangement of postcloacal pairs are similar to each other, *i.e.*, a subventral pair occurs around one CBD posterior to CO, a lateral pair occurs around the mid-tail and two tiny ventral pairs and phasmids occur at just anterior to the tail tip (Poinar & Herre, 1991). This pattern is also conserved in subgroup 1 (Kanzaki *et al.*, 2010) and is considered as the common apomorphic (= plesiomorphic) character of the clade. Furthermore, except for subgroup 2, the *P. laevigata* group species share relatively short and stout spicules. Contrastingly, the *P. maxinema* group has six pairs of postcloacal papillae including a distal dorsal pair and a long and slender spicule (Kanzaki *et al.*, 2013, 2014b), the *P. australis* group has a large and arcuate spicule and a very short tail with four pairs including a distal dorsal pair (*P. salicifoliae*) or five pairs (*P. australis*) (Bartholomaeus *et al.*, 2009; Wöhr *et al.*, 2015), and *P. sycophilon* has a short and stout spicule and five pairs of postcloacal

papillae including a dorsal distal pair (Wöhr *et al.*, 2014). The *P. australis* group also has the autapomorphy of a single prodelphic ovary, which might be a factor in the evolution of male tail or copulatory characters or mating behaviour.

Although the stoma and male tail characters of subgroups 2 and 3 are similar to each other, spicule morphology between these subgroups is divergent. The spicule of *P. obtusinema* has a relatively long, slender and ventrally arcuate blade, and that of *P. nymphanema* has a large manubrium and is ventrally bent with a distally tapered blade. Further, the gubernaculum of subgroup 2 appears slightly elongated compared with the other species in the *P. laevigata* group (Poinar & Herre, 1991; Giblin-Davis *et al.*, 2006). Similarly, the spicule and gubernaculum morphologies were more or less variable among the morphospecies in *P. maxinema* group (Kanzaki *et al.*, 2014b). Although the details of functional adaptations, *e.g.*, the relationship between spicule and gubernaculum morphologies and mating behaviour, are unknown, these relatively plastic characters could be useful for characterising species.

In the present and previous studies, although the qualitative characters were basically identical within each species, quantitative characters, especially body size varied among individuals. For example, the male body length of *P. obtusinema* ranged from 1986 to 3594 μm (present study) or 1920 to 3990 μm (previous study: Poinar &

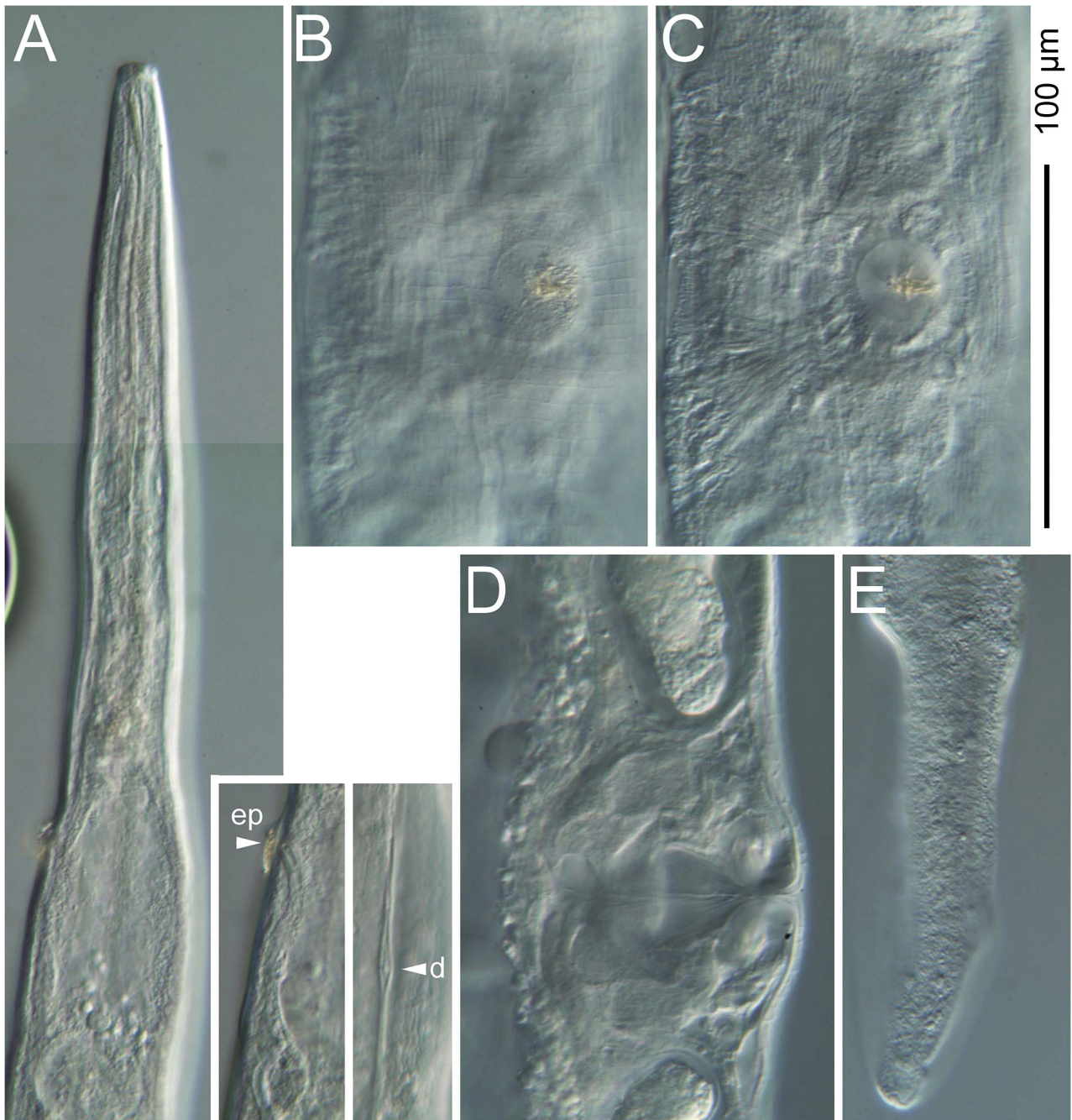


Fig. 11. Adult female of *Parasitodiplogaster nymphanema*. A: Neck region showing excretory-secretory system (ep) and deirid (d); B, C: Ventral view of vulval region in different focal planes; D: Lateral view of vulval region; E: Tail. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/15685411>.

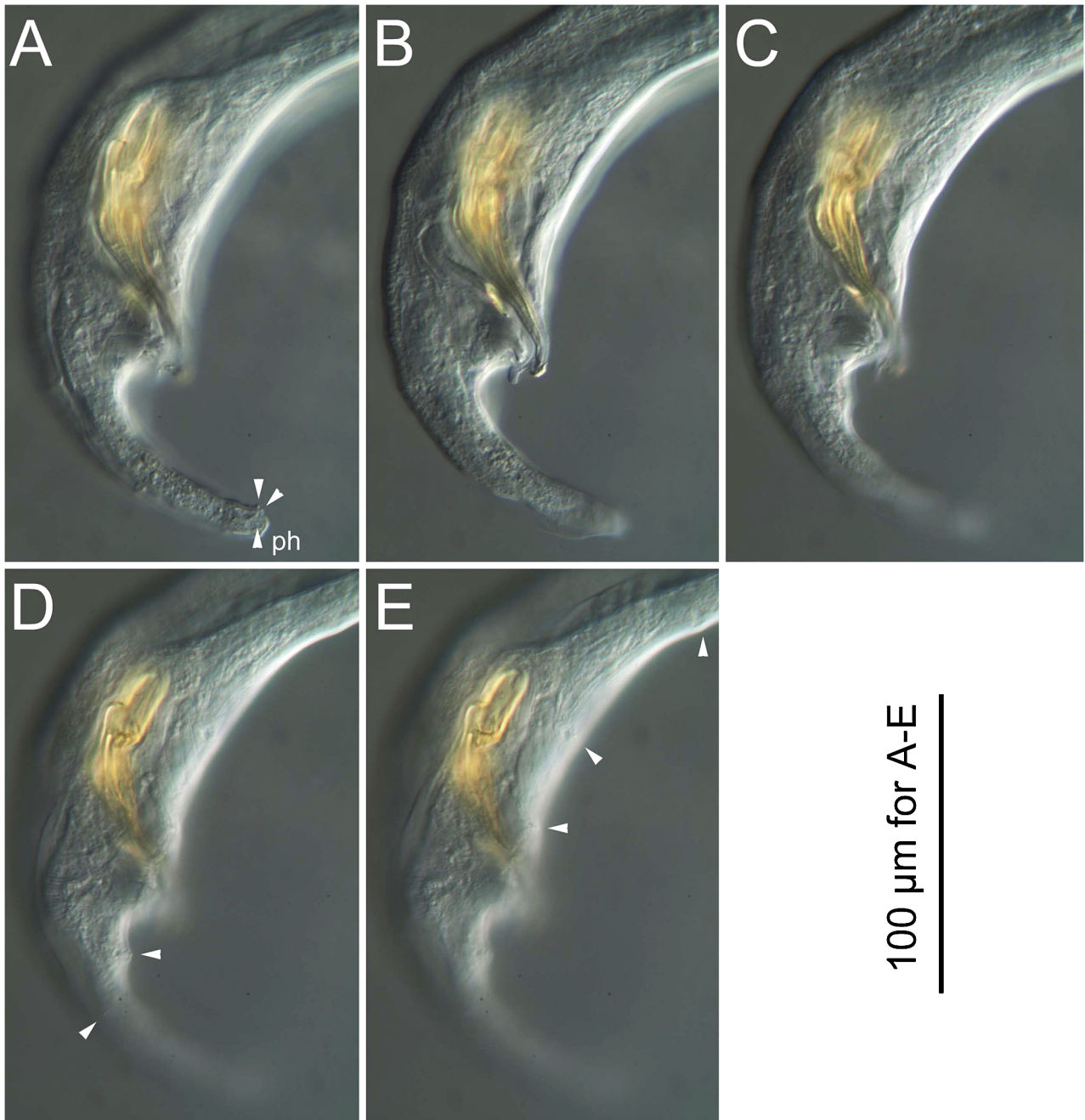


Fig. 12. Adult male tail of *Parasitodiplogaster nymphanema*. A-E: Tail region in different focal planes showing second to eighth pairs of genital papillae (arrowheads) and phasmid (ph) (first pair of genital papillae is far anterior to cloacal opening). This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/15685411>.

Table 4. Morphometric values of *Parasitodiplogaster nymphanema*. All measurements are in μm . Values obtained in the present study are in the form: mean \pm s.d. (range), and those from the original description: mean (range).

Character	Male		Female	
	Present study	Original description	Present study	Original description
n	10	10	10	10
L	2381 \pm 544 (1782-3304)	1150 (960-1520)	2946 \pm 387 (2348-3551)	2136 (1560-2880)
a	25.0 \pm 3.4 (19.5-28.8)	Not given	23.2 \pm 2.9 (19.2-28.5)	Not given
b	10.8 \pm 1.5 (9.0-12.8)	Not given	13.1 \pm 1.1 (11.5-15.6)	Not given
c	21.8 \pm 3.1 (17.5-27.4)	Not given	19.5 \pm 1.2 (18.4-21.5)	Not given
c'	1.6 \pm 0.2 (1.3-2.1)	Not given	3.3 \pm 0.5 (2.6-4.0)	Not given
T or V	55.6 \pm 11.6 (35.7-70.8)	Not given	49.6 \pm 0.9 (48.1-50.7)	50 (44-63)
Anterior pharynx length	137 \pm 15 (117-161)	120 (102-141)	146 \pm 11 (125-159)	116 (78-135)
Posterior pharynx length	75 \pm 12 (51-94)	68 (42-90)	73 \pm 8.2 (64-90)	72 (60-84)
Anterior/posterior pharynx ratio	1.87 \pm 0.4 (1.58-2.94)	1.79 (1.43-2.44)	2.02 \pm 0.2 (1.68-2.50)	1.64 (1.45-1.79)
Nerve ring from anterior end	179 \pm 19 (158-216)	137 (117-156)	190 \pm 12 (106-203)	137 (96-159)
Excretory pore from anterior end	236 \pm 35 (199-301)	158 (78-210)	263 \pm 26 (232-314)	197 (135-273)
Excretory pore from basal bulb	18 \pm 17 (0-41)	Not given	38 \pm 20 (10-87)	Not given
Max. body diam.	99 \pm 35 (68-148)	76 (60-92)	130 \pm 27 (96-180)	96 (75-129)
Vulval body diam.	–	–	126 \pm 25 (96-180)	Not given
Testis length	1353 \pm 486 (689-1946)	Not given	–	–
Anterior ovary length	–	–	606 \pm 216 (368-1018)	Not given
Posterior ovary length	–	–	611 \pm 197 (368-959)	Not given
Cloacal or anal body diam.	69 \pm 11 (55-83)	56 (51-64)	47 \pm 8.2 (33-59)	35 (24-48)
Tail length	109 \pm 17 (84-133)	84 (71-105)	151 \pm 21 (125-193)	132 (75-183)
Spicule length (chord) ¹	98 \pm 8.6 (88-117)	78 (66-84)	–	–
Spicule length (curve) ²	112 \pm 9.8 (104-135)	Not given	–	–
Gubernaculum length	39 \pm 3.5 (35-46)	28 (22-33)	–	–

¹ Spicule length in chord from anterior end of manubrium and distal end.² Spicule length curved along middle part from anterior end of manubrium to distal end.

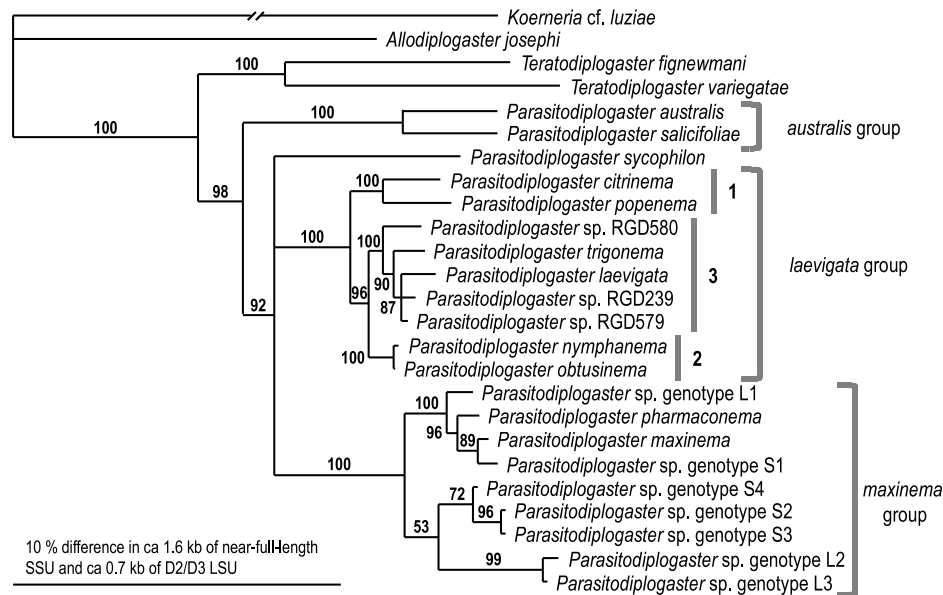


Fig. 13. Phylogenetic relationships among *Parasitodiplogaster* spp. Combined Bayesian tree inferred from near-full-length of SSU and D2-D3 expansion segments of LSU ribosomal RNA genes. The substitution models for SSU and LSU are GTR + I + G (AIC = 9750.1973; lnL = 4865.0986; freqA = 0.2541; freqC = 0.2088; freqG = 0.2694; freqT = 0.2677; R(a) = 1.3880; R(b) = 3.3455; R(c) = 2.8751; R(d) = 0.8373; R(e) = 8.7753; R(f) = 1.0000; Pinvar = 0.4403; Shape = 0.7224) and TrN + G (AIC = 9845.1807; lnL = 4916.5903; freqA = 0.1895; freqC = 0.2170; freqG = 0.3383; freqT = 0.2552; R(a) = 1.0000; R(b) = 2.0928; R(c) = 1.0000; R(d) = 1.0000; R(e) = 6.4254; R(f) = 1.0000; Pinvar = 0; Shape = 0.4647), respectively. Posterior probability values exceeding 50% are given on appropriate clades.

Herre, 1991), *i.e.*, the largest individual was about twice as long as the smallest. The factor(s) affecting such large variation was (were) not examined in the present study, but the age of figs, humidity, microbial flora and other factors could affect this variability. More physiological studies, *e.g.*, feeding habits of the adult nematodes (Ramírez-Benavides & Salazar-Figueroa, 2015), will be necessary for better understanding of their life history strategy.

Lastly, comparisons of the general host plant usage and geographical patterns of the main nematode groupings in Figure 13 suggest that *Teratodiplogaster* and the clades of *Parasitodiplogaster* all shared a common fig- and fig wasp-associated ancestor and have since radiated along major *Ficus* lineages. The *Teratodiplogaster* clade appears to be restricted to the *Ficus* subgenus *Sycomorus* and section *Sycomorus* in Australasia and Africa, the *P. australis* group appears to be confined to the subgenus *Urostigma*, section *Urostigma* figs in Australasia and Africa, the *P. sycophilon* group appears to be associated with the subgenus *Urostigma*, section *Galoglychia* figs of Africa, the *P. laevigata* group appears to be restricted to the subgenus *Urostigma*, section *Americana*

figs in the Americas, and the *P. maxinema* group appears to be associated with the subgenus *Pharmacosycea*, section *Pharmacosycea* figs from the Americas. Further studies of this interesting fig- and fig wasp-associated diplogastrid lineage should help elucidate how stomatal and reproductive morphology manifest under differing microhabitat evolutionary challenges and how this plays out in nematode speciation in specialised associations with insects and plants.

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References

- Bartholomaeus, F., Davies, K.A., Ye, W., Kanzaki, N. & Giblin-Davis, R.M. (2009). *Schistonchus virens* sp. n. (Aphelenchoi-
didae) and *Parasitodiplogaster australis* sp. n. (Diplogastri-
dae) from *Ficus virens* (Moraceae) in Australia. *Nematology*
11, 583-601.
- Davies, K.A., Ye, W., Giblin-Davis, R.M. & Thomas, W.K.
(2008). *Ficotylus congestae* gen. n., sp. n. (Anguinata), from
Ficus congesta (Moraceae) sycones in Australia. *Nematology*
11, 63-75.
- Davies, K.A., Bartholomaeus, F., Kanzaki, N., Ye, W. & Giblin-
Davis, R.M. (2015). A review of the taxonomy, phylogeny,
distribution and co-evolution of *Schistonchus* (Nematoda:
Aphelenchoiidae) and proposal of *Ficophagus* n. gen. and
Martininema n. gen. *Nematology* 17, 761-829.
- Giblin-Davis, R.M., Ye, W., Kanzaki, N., Williams, D., Morris,
K. & Thomas, W.K. (2006). Stomatal ultrastructure, molec-
ular phylogeny and description of *Parasitodiplogaster lae-
vigata* sp. n. (Nematoda: Diplogastriidae), a parasite of fig
wasps. *Journal of Nematology* 38, 137-149.
- Harrison, R.D. (2005). Figs and the diversity of tropical rain-
forests. *Bioscience* 55, 1053-1064.
- Huelsenbeck, J.P. & Ronquist, F. (2001). MRBAYES: Bayesian
inference of phylogenetic trees. *Bioinformatics* 17, 754-755.
- Kanzaki, N. & Futai, K. (2002). A PCR primer set for determina-
tion of phylogenetic relationships of *Bursaphelenchus* species
within the *xylophilus* group. *Nematology* 4, 35-41.
- Kanzaki, N., Giblin-Davis, R.M., Davies, K., Ye, W., Center,
B.J. & Thomas, W.K. (2009). *Teratodiplogaster fignewmani*
gen. nov., sp. nov. (Nematoda: Diplogastriidae) from the
syconia of *Ficus racemosa* in Australia. *Zoological Science*
26, 569-578.
- Kanzaki, N., Giblin-Davis, R.M., Herre, E.A. & Center, B.J.
(2010). Redescription of two Panamanian nematodes, *Para-
sitodiplogaster citrinema* Poinar & Herre, 1991 and *P. popen-
ema* Poinar & Herre, 1991. *Nematology* 12, 89-104.
- Kanzaki, N., Giblin-Davis, R.M., Davies, K.A. & Center, B.J.
(2012). *Teratodiplogaster martini* n. sp. and *Parasitodiplo-
gaster doliostoma* n. sp. (Nematoda: Diplogastriidae) from the
syconia of *Ficus* species from Africa. *Nematology* 14, 529-
546.
- Kanzaki, N., Giblin-Davis, R.M., Ye, W., Herre, E.A. & Cen-
ter, B.J. (2013). Description of *Parasitodiplogaster pharma-
conema* n. sp. and redescription of *P. maxinema* from *Ficus*
maxima Mill. (Moraceae). *Nematology* 15, 957-974.
- Kanzaki, N., Tanaka, R., Giblin-Davis, R.M. & Davies, K.
(2014a). New plant-parasitic nematode from the mostly
mycophagous genus *Bursaphelenchus* discovered inside figs
in Japan. *PLoS ONE* 9, e99241.
- Kanzaki, N., Giblin-Davis, R.M., Ye, W., Herre, E.A. & Center,
B.J. (2014b). *Parasitodiplogaster* species associated with
Pharmacosycea figs in Panama. *Nematology* 16, 607-619.
- Katoh, K., Misawa, K., Kuma, K. & Miyata, T. (2002). MAFFT:
a novel method for rapid multiple sequence alignment based
on fast Fourier transform. *Nucleic Acids Research* 30, 3059-
3066.
- Körner, H. (1954). Die Nematodenfauna des vergehenden
Holzes und ihre Beziehungen zu den Insekten. *Zoologische*
Jahrbucher (Systematik) 82, 245-353.
- Large, B. & Simon, D.L. (1999). Markov chain Monte Carlo
algorithms for the Bayesian analysis of phylogenetic trees.
Molecular Biology and Evolution 16, 750-759.
- Meyl, A.H. (1960). Die freilebenden Erd- und Süßwassernema-
toden (Fadenwürmer). In: Brohmer, P., Ehrmann, P. & Ulmer,
G. (Eds). *Die Tierwelt Mitteleuropas, Vol. 1 (5a)*. Leipzig,
Germany, Quelle & Meyer.
- Poinar Jr, G.O. (1979). *Parasitodiplogaster sycophilus* gen.
n., sp. n. (Diplogasteridae: Nematoda), a parasite of *Elis-
abethiella stuckenbergi* Grandi (Agaonidae: Hymenoptera)
in Rhodesia. *Proceedings of the Koninklijke Nederlandse*
*Akademie van Wetenschappen, Series C: Biological and Med-
ical Sciences* 82, 375-381.
- Poinar Jr, G.O. & Herre, E.A. (1991). Speciation and adap-
tive radiation in the fig wasp nematode, *Parasitodiplogaster*
(Diplogasteridae: Rhabditida) in Panama. *Revue de Néma-
tologie* 14, 361-374.
- Posada, D. & Crandall, K.A. (1998). MODELTEST: testing the
model of DNA substitution. *Bioinformatics* 14, 817-818.
- Ramírez-Benavides, W. & Salazar-Figueroa, L. (2015). *Para-
sitodiplogaster citrinema* is an internal necrophagous species
of the pollinating fig wasp *Pegoscapus tonduzi*. *Nematology*
17, 733-738.
- Southey, J.F. (Ed.) (1970). *Laboratory methods for work with*
plant and soil nematodes. London, UK, Her Majesty's Sta-
tionery Office.
- Sudhaus, W. & Fürst von Lieven, A. (2003). A phylogenetic
classification and catalogue of the Diplogastriidae (Secern-
tea, Nematoda). *Journal of Nematode Morphology and Sys-
tematics* 6, 43-89.
- Susoy, V., Ragsdale, E.J., Kanzaki, N. & Sommer, R.J. (2015).
Rapid diversification associated with a macroevolutionary
pulse of developmental plasticity. *eLife* 4, e05463.
- Susoy, V., Herrmann, M., Kanzaki, N., Kruger, M., Nguyen,
C.N., Rödelserperger, C., Röseler, W., Weiler, C., Giblin-Davis,
R.M., Ragsdale, E.J. et al. (2016). Large-scale diversification
without genetic isolation in nematode symbionts of figs.
Science Advances 2, e1501031.
- Tanaka, R., Kikuchi, T., Aikawa, T. & Kanzaki, N. (2012).
Simple and quick methods for nematode DNA preparation.
Applied Entomology and Zoology 4, 291-294.
- Williams, B.D., Schrank, B., Huynh, C., Shownkeen, R. & Wa-
terson, R.H. (1992). A genetic mapping system in *Caeno-
rhabditis elegans* based on polymorphic sequence tagged
sites. *Genetics* 131, 609-624.
- Wöhr, M., Greeff, J.M., Kanzaki, N., Ye, W. & Giblin-Davis,
R.M. (2014). Molecular and morphological observations

- on *Parasitodiplogaster sycophilon* Poinar, 1979 (Nematoda: Diplogastrina) associated with *Ficus burkei* in Africa. *Nematology* 16, 453-462.
- Wöhr, M., Greeff, J.M., Kanzaki, N. & Giblin-Davis, R.M. (2015). *Parasitodiplogaster salicifoliae* n. sp. (Nematoda: Diplogastrina) from *Ficus ingens* and *F. salicifolia* in South Africa. *Nematology* 17, 301-311.
- Ye, W., Giblin-Davis, R.M., Braasch, H., Morris, K. & Thomas, W.K. (2007). Phylogenetic relationships among *Bursaphelenchus* species (Nematoda: Parasitaphelenchidae) inferred from nuclear ribosomal and mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution* 43, 1185-1197.
- Yoder, M., De Ley, I.T., King, I.W., Mundo-Ocampo, M., Mann, J., Blaxter, M., Poiras, L. & De Ley, P. (2006). DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. *Nematology* 8, 367-376.