

Allodiplogaster josephi n. sp. and *A. seani* n. sp. (Nematoda: Diplogastridae), associates of soil-dwelling bees in the eastern USA

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Summary – Two commensal associates of bees, *Allodiplogaster josephi* n. sp. from the Dufour's gland of a cellophane bee (*Colletes thoracicus*) from Maryland, USA, and *A. seani* n. sp. from the abdominal glands of an andrenid bee (*Andrena alleghaniensis*) from New York, USA, are described and illustrated. Both species were collected as dauers from their respective hosts and cultured on bacteria on tryptic soy broth (TSB) or NGM agar. *Allodiplogaster josephi* n. sp. and *A. seani* n. sp. are morphologically closer to each other than to other species of *Allodiplogaster*, which was recently revised to include 37 valid species. However, the two new species are distinguished by reproductive isolation, shape of the spicule manubrium, host associations and molecular characters, the latter in sequences of the near-full length small subunit (SSU) rRNA gene, D2-D3 expansion segments of the large subunit (LSU) rRNA gene and partial mitochondrial COI. Morphological characterisation was supplemented by scanning electron microscopy (SEM), which revealed furcation of both v5 and v6 male genital papillae, consistent with previous reports for species of the *henrichae* group of *Allodiplogaster*.

Keywords – *Andrena alleghaniensis*, Andrenidae, *Colletes thoracicus*, Colletidae, description, molecular, morphology, morphometrics, new species, phoresy, phylogeny, taxonomy.

Soil-dwelling bees from several families are known as phoretic hosts of different genera of free-living microbivorous nematodes (Altenkirch, 1962; Giblin *et al.*, 1981, 1984; Giblin & Kaya, 1983, 1984; Giblin-Davis *et al.*, 1990, 1993, 2005, 2010; Kanzaki, 2006; Hazir *et al.*, 2007; McFrederick & Taylor, 2013). In particular, members of two genera of diplogastrid nematodes have evolved in close association with their bee hosts: *Acrostichus* Rahm, 1928 (= *Aduncospiculum* Giblin & Kaya, 1984), which includes phoretic associates of some genera of sweat bees (Halictidae Thomson) (Giblin-Davis *et al.*, 1990; Kanzaki *et al.*, 2010a, b; McFrederick & Taylor, 2013), and *Allodiplogaster* Paramonov & Sobolev in Skrjabin, Shikobalova, Sobolev, Paramonov & Sudarikov, 1954 (= *Koerneria* Meyl, 1960), which includes associates of bees of Andrenidae Latreille and Colletidae Lepeletier (Batra, 1980; Giblin-Davis *et al.*, 1990). Additionally, the diplogastrid nematode *Pristionchus mau-*

pasi (Potts, 1910) Paramonov, 1952 was recently recovered from *Andrena optata* Warncke from Turkey, but this species is hypothesised to have relatively wide host association preferences (Hazir *et al.*, 2015).

An unidentified diplogastrid dauer, possibly belonging to the genus *Allodiplogaster*, was the first recorded non-parasitic nematode from soil-dwelling bees and was reported from the abdominal glands of *Andrena vaga* Panzer, *A. fulva* Schrank and *A. albicans* Müller females in Germany (Alterkirch, 1962). Since then, a species of *Allodiplogaster* (= *Koerneria*) was isolated and cultured (RGD228) from the abdominal glands of the andrenid bee *Andrena alleghaniensis* Viereck from New York State, USA (Giblin-Davis *et al.*, 1990). Batra (1980) reported unidentified juvenile nematodes from the Dufour's glands of *Colletes* cellophane bees from Maryland, USA, which were later re-isolated from *C. thoracicus* Smith, cultured (RGD227), and confirmed to be a species of *Allodiplo-*

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gaster (= *Koerneria*) (Giblin-Davis *et al.*, 1990). These nematode isolates were determined to be two new species and are described here based upon their morphological and molecular relationships to other species of the newly resurrected *henrichae* group of *Allodiplogaster* (Kanzaki *et al.*, 2014a).

Materials and methods

NEMATODE ISOLATION

Type cultures of nematodes were previously collected as described by Giblin-Davis *et al.* (1990). In brief, an adult female of *C. thoracicus* from Prince George's County, Maryland, USA, was dissected on 19 May 1986 and dauer nematodes from the Dufour's gland were transferred to 10% tryptic soy broth (TSB) agar to establish the type culture RGD227, which has since been maintained in the lab with biweekly subculturing at room temperature using *Escherichia coli* strain OP50 or unidentified bacteria as a food source. Type culture RGD228 was established on 20 June 1987 from dauer nematodes from the excised and ablated abdominal glands of a female of *Andrena alleghaniensis* from the central Adirondacks near Saranac Lake, Franklin County, New York, USA, on TSB and maintained as above.

MORPHOLOGICAL OBSERVATION AND MORPHOMETRICS

Nematodes were subcultured to NGM agar seeded with *E. coli* OP50 to standardise the food source with that of other diplogastrid species kept in laboratory culture, and were kept at room temperature. One- to 4-week-old cultures of both species were used for morphological observations, drawings, light micrographs and type specimens.

To prepare type material, a small amount (*ca* 5–10 ml) of M9 buffer (3.0 g KH₂PO₄; 6.0 g Na₂HPO₄; 0.5 g NaCl; 1.0 g NH₄Cl; and *quantum satis* to 1 litre with distilled water) was pipetted onto each culture plate to allow collection of suspended nematodes into small test tubes. The nematode suspension was allowed to settle for 10–15 min, decanted to reduce the volume to *ca* 1 ml, and the suspended nematodes were heat-killed at 60°C and fixed in TAF (7.0% formalin; 2.0% triethanolamine; 91% distilled water). After 48 h of fixation, nematodes were dehydrated through a glycerin-ethanol series using a modified Seinhorst's method (Minagawa & Mizukubo, 1994), and mounted in glycerin according to the methods

of Maeseneer & d'Herde (described in Hooper, 1986). Mounted type specimens were used for morphometrics with the aid of a drawing tube connected to an Eclipse 80i (Nikon, Tokyo, Japan) light microscope.

The eury stomatous morphs of both species were usually rare in culture and were mostly observed in older (3- to 4-week-old) cultures. Additionally, the eury stomatous morph of the new species could also be induced by adding large numbers of juveniles of *Caenorhabditis elegans* Maupas as a food source on non-nutritional water agar plates, as described by Susoy *et al.* (2015). Nematodes were washed from plates with M9 buffer into a Syracuse watch glass and eury stomatous individuals were hand-picked from the nematode suspension using a fine stainless steel wire pick under an S8Apo (Leica) dissecting microscope. Eury stomatous individuals were mounted on permanent slides for further observation as described above.

For drawings, live specimens were mounted into water or M9 buffer on slides with silicon grease and then relaxed by applying gentle heat (Kanzaki, 2013). Stomatal morphology was analysed by pressing the head region to evert and separate stomatal structures (Kanzaki, 2013). Light micrographs were taken with a microscopic digital camera system DS-Ri1 (Nikon) connected to the light microscope and then annotated for figures using Photoshop Elements v. 9 (Adobe).

SCANNING ELECTRON MICROSCOPY

External morphology of the adults of both sexes of the two new species was examined by scanning electron microscopy (SEM). Specimens were fixed in 3% (v/v) glutaraldehyde, post-fixed in 2% OsO₄ for 12 h at 22°C, rinsed in dH₂O, dehydrated in a graded ethanol series, critical point dried from liquid CO₂, mounted on a stub with double-stick tape, sputter-coated with 20 nm of gold-palladium, and viewed with a Hitachi S-4000 Field Emission SEM operating at 7 kV.

MOLECULAR PHYLOGENETIC ANALYSIS

The molecular sequences of a near-full-length fragment of the small subunit ribosomal RNA gene (SSU, 18S) (AB440306, LC027671), D2-D3 expansion segments of the large subunit ribosomal RNA gene (LSU, 28S) (AB440321, AY840563), and a fragment of the mitochondrial cytochrome *c* oxidase subunit I (mtCOI) (AB501141, AB501142) of the two new species were previously available in the GenBank database, except for

the SSU of RGD228, which was obtained in the present study. Most of the included sequences were used in previous phylogenetic analyses of Diplogastridae (e.g., Kanzaki *et al.*, 2009a, 2010a, 2011a, 2012a, 2014a; Atighi *et al.*, 2013; Kanzaki & Giblin-Davis, 2014), leading to inferences similar to those presented herein. The molecular phylogenetic status of the two new species was inferred from SSU sequences using Bayesian, maximum likelihood (ML), and maximum parsimony (MP) analyses.

Operational taxonomic units (OTUs) representing putative species were compared with species included in previous studies of the phylogeny of Diplogastridae (e.g., Mayer *et al.*, 2007; Kanzaki & Giblin-Davis, 2014). *Rhabditoides inermis* (Schneider, 1866) Dougherty, 1955 was used as an outgroup species, as informed by phylogenetic inferences of rhabditid nematodes performed by Kiontke *et al.* (2007) and Sudhaus (2011). Sequences were aligned using MAFFT (Katoh *et al.*, 2002). The best-fitting base substitution model under the Akaike information criterion (AIC) was determined to be GTR + I + G in Model-Test version 3.7 (Posada & Crandall, 1998). The Akaike-supported model, log likelihood (lnL), AIC values, proportion of invariable sites, gamma distribution shape parameters, and substitution rates were used in the analyses. Bayesian inference was performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). Four chains were run for 4×10^6 generations, and 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. The online version of PhyML 3.0 (Guindon *et al.*, 2010) was employed for the ML analysis. Empirical parameters obtained during model selection procedure were used in the ML analysis. An unweighted MP analysis was performed using PHYLIP 3.69 (Felsenstein, 2005) with default settings. The tree topologies obtained from ML and MP analyses were evaluated with 1000 bootstrap pseudoreplicates.

MATING TESTS

Hybrid crosses were performed to test for reproductive isolation between the putative new *Allodiplogaster* species. In addition to the two strains from bees, mating tests included a third strain, *Allodiplogaster* sp. RS1982 (Mayer *et al.*, 2007), isolated from cockchafer in Europe but which showed close molecular identity to the former strains (see below). All crosses were performed

on plates seeded with small bacterial lawns of approximately the same diameter, as grown from 25 μ l *E. coli* OP50 in L-broth. Three types of crosses were performed: i) *Allodiplogaster* sp. RGD227 \times *Allodiplogaster* sp. RGD228; ii) *Allodiplogaster* sp. RGD227 \times *Allodiplogaster* sp. RS1982; and iii) *Allodiplogaster* sp. RGD228 \times *Allodiplogaster* sp. RS1982. Each cross consisted of three virgin (J4) females of one strain and five males of the opposite strain. Crosses were performed reciprocally and in at least three replicates for each direction. As a control, self-crosses (*i.e.*, within strains) were performed between three virgin females and five males placed under the same conditions as for crosses between strains. All crosses were checked for eggs and motile offspring for 2 weeks, unless viable offspring were produced within a shorter time period.

For crosses that resulted in viable F1 offspring, these F1 were self-crossed to determine whether they were self-fertile and thus biologically conspecific. In F1 crosses, five virgin females were mated to five males under the same conditions as for P0 crosses.

Results

MOLECULAR PHYLOGENETIC ANALYSIS

Tree topologies inferred by Bayesian, ML and MP analyses were congruent except for differences in terminal nodes outside of *Allodiplogaster*. Because relationships among *Allodiplogaster* species were identical across trees, only the Bayesian tree is shown, with bootstrap support values from ML and MP analyses added to the tree.

The new species, together with *A. lucani* (Körner, 1954) Kanzaki, Ragsdale & Giblin-Davis, 2014a, and two other undescribed or unidentified strains of *Allodiplogaster* were inferred to form a well-supported clade, with 100% posterior probability (PP) (Fig. 1). Within the *Allodiplogaster* clade, *A. lucani* is clearly separated from a well-supported (100% PP) clade of all other sequenced *Allodiplogaster* isolates: *Allodiplogaster* sp. RGD227, *Allodiplogaster* sp. RGD228, *Allodiplogaster* sp. RS1982, and two other undescribed or unidentified strains of *Allodiplogaster* (*Allodiplogaster* sp. SB110 and *Allodiplogaster* sp. KoerSp2), whereby *Allodiplogaster* sp. RGD227 is a sister taxon to all other isolates in that clade. *Allodiplogaster* sp. RGD228 and *Allodiplogaster* sp. RS1982 showed a difference of 3 bp in the *ca* 1.6 kb SSU sequence. Sequence differences among the other three isolates of the latter clade, and between those iso-

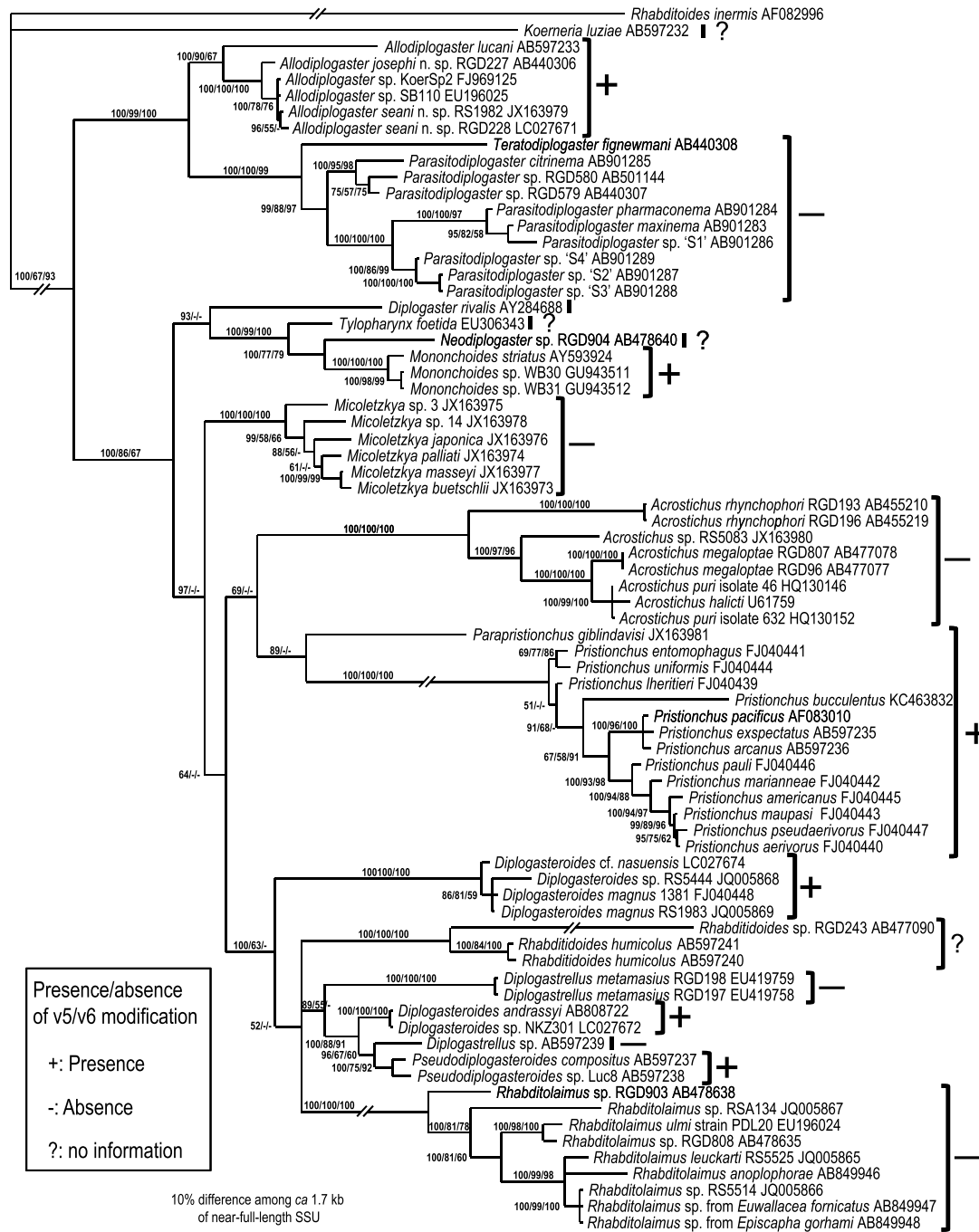


Fig. 1. Phylogenetic tree inferred by Bayesian analysis of near-full-length sequences of the small subunit ribosomal RNA gene under a GTR + I + G model (lnL = 16 808.3906; freqA = 0.2396; freqC = 0.2077; freqG = 0.2686; freqT = 0.2841; R(a) = 1.2571; R(b) = 2.9775; R(c) = 2.4171; R(d) = 0.5044; R(e) = 5.5076; R(f) = 1; Pinvar = 0.3961; Shape = 0.5573). Tree topologies inferred by Bayesian, maximum likelihood (ML), and maximum parsimony (MP) analyses were largely congruent, including identical relationships among *Allodiplogaster* species. Posterior probability (middle value) or bootstrap support (right value) values exceeding 50% are given on the Bayesian tree.

lates and *Allodiplogaster* sp. RGD228 or *Allodiplogaster* sp. RS1982, were 1-4 bp in the analysed SSU sequence. Therefore, these strains were hypothesised to belong to a single species. Although the lack of access to morphological vouchers for *Allodiplogaster* sp. SB110 and *Allodiplogaster* sp. KoerSp2 precludes the definitive naming of these strains, we tested for biological (reproductive) isolation among the other three previously unnamed strains.

MATING TESTS

Crosses between *Allodiplogaster* strains revealed them to belong to two reproductively compatible groups. Crosses between *Allodiplogaster* sp. RGD227 and either of the other two strains did not result in any viable offspring, although many eggs containing dead embryos were observed in reciprocal crosses of *Allodiplogaster* sp. RGD227 and *Allodiplogaster* sp. RGD228. In contrast, crosses between *Allodiplogaster* sp. RGD228 and *Allodiplogaster* sp. RS1982 in both directions resulted in F1 offspring that produced fully viable F2 when selfed. Therefore, we consider the latter two strains to belong to a single biological species that is reproductively isolated from *Allodiplogaster* sp. RGD227.

Taxonomic descriptions

Reflecting their inferred phylogenetic closeness, the two new *Allodiplogaster* species share many morphological characters. To avoid redundancy, characters common to both species are described first, followed by species-specific characters and diagnoses for each species.

DESCRIPTION OF COMMON CHARACTERS

Adults

Body size varying between individuals, presumably due to fluctuating cultural conditions over a long time period (1-4 weeks after subculturing). Eurystomatous morphs generally smaller than stenostomatous individuals in body length, perhaps due to effects of starvation pressure on eurystomatous morph induction (Bento *et al.*, 2010) and growth capacity. Body cylindrical, stout. Cuticle thick, with fine annulation and clear longitudinal striations. Anterior boundary of longitudinal striation at level of posterior end of stoma. Lateral field consisting of two lines, weakly distinguishable from body striations by their relation to deirid position. Head without apparent lips, *i.e.*, lip sectors only weakly separated

from one another, no clear constriction being observed between lip region and remainder of body, and with six short and papilliform labial sensilla. Four small, papilliform cephalic papillae present in male, as typical for diplogastrid nematodes. Amphidial apertures on lateral lip sectors at level of posterior end of cheilostomatal plates, oval-shaped, slightly dorsally shifted from midline. Stomatal dimorphism present (described in detail below). Dorsal pharyngeal gland clearly observed, penetrating dorsal tooth with narrow duct. Anterior part of pharynx (= pro- and metacarpus) *ca* 1.3 times as long as posterior part (isthmus and basal bulb). Inner wall (lumen) of anterior pharynx well cuticularised, lining appearing reflective in light microscopy, bearing many fishbone-like, sclerotised projections. Clear separation of lining between procorpus and metacarpus observed. Procorpus very muscular, stout, occupying two-thirds or more of corresponding body diam. Metacarpus very muscular, well developed, isthmus narrow, not muscular. Basal bulb glandular. Pharyngo-intestinal junction clearly observed, well developed. Nerve ring usually surrounding middle or slightly more posterior part of isthmus. Excretory pore not conspicuous, of variable position, mostly at level of basal bulb and pharyngo-intestinal junction or sometimes slightly posterior in stenostomatous individuals, and at level of isthmus (between nerve ring and basal bulb) in eurystomatous individuals. Excretory duct perpendicular to body axis, turning and extending posteriorly. Hemizonid barely visible by light microscopy (LM). Deirid lateral, slightly posterior to pharyngo-intestinal junction. Postdeirid observed clearly in female but not in male. Posterior part of intestine forming pre-rectum, *i.e.*, having a clear constriction and distinguished from main, anterior part of intestine. Pre-rectum present in both sexes, but not clearly observed in male as intestine sometimes distorted by testis (*vas deferens*), thus making constriction vague.

Stomatal morphology (stenostomatous form)

Cheilostom consisting of at least six per- and inter-radial plates, sometimes with one or two plates splits into two narrower plates. Each plate with weak longitudinal striations. Incisions between plates sometimes obfuscated by longitudinal striation and not easily distinguished by LM. Gymnostom short, cuticular, ring-like, slightly narrowing to anterior end with smooth edge. Anterior end (*ca* 20% of total length) overlapping cheilostom medially (internally). Dorsal gymnostomatal wall slightly thickened compared with ventral side. Stegostom separated into three parts: pro- + mesostegostom, metastegostom, and telostegostom. Pro- + mesostegostom weakly

cuticularised, ring-like, surrounding anterior end of pharynx. Metastegostom bearing two teeth: a claw-like dorsal tooth and long and triangular right subventral tooth. Right subventral sector bearing narrow claw-like tooth with strongly cuticularised surface and curve-shaped. Left subventral sector with three denticles on lateral, subventral and ventral sides. Dorsal telostegostomatal wall strongly sclerotised, posterior end forming two pointed projections (struts) extending to anterior end of pharynx. Ventral stegostomatal wall strongly sclerotised, with two rounded subventral apodemes in posterior part of stoma.

Stomatal morphology (eurystomatous form)

Cheilostom consisting of at least six per- and interradial plates, sometimes with one or two plates split into two narrower plates. Plates wider than those of stenostomatous form, such that posterior end almost as wide as height of plate. Each plate with weak longitudinal striations. Separation between plates clearly observed by LM. Anterior end of each plate flattened or with irregular, wavy pattern. Gymnostom short, cuticular, ring-like, slightly narrowing to anterior end. Anterior half overlapping cheilostom medially (internally). Anterior end of gymnostomatal ring serrated. Dorsal gymnostomatal wall slightly thickened compared with ventral side. Stegostom separated into three parts: pro- + mesostegostom, metastegostom, and telostegostom. Pro- + mesostegostom ring-like, weakly cuticularised, surrounding anterior end of pharynx. Metastegostom bearing two teeth: claw-like dorsal tooth and claw-like right subventral tooth. Left subventral sector bearing three plates on lateral, subventral, and ventral sides, whereby lateral plate larger than other two. Detailed morphology of plates described below for each species. Dorsal telostegostomatal wall strongly sclerotised, with posterior end forming two pointed projections (struts) extending into anterior end of pharynx. Ventral stegostomatal wall strongly sclerotised, with two large, rounded, and quadrilateral subventral apodemes in posterior part of stoma.

Male

Ventrally arcuate, strongly ventrally curved at tail region when killed by heat. Testis single, lying along ventral side of body, anterior part reflexed to right-hand side. Posterior part of testis slightly less than half of total gonad length, forming *vas deferens*, latter sometimes containing a few sperm. *Vas deferens* distinguished from testis by size and arrangement of its cells, such that *vas deferens* composed of relatively large, flattened cells. Three

(two subventral and one dorsal) gland cells opening into cloaca. Spicules paired, separate. Detailed morphology of spicules and gubernaculum described for each species below. Cloacal opening slit-like in ventral view, with anterior cloacal lip extending slightly to cover cloacal opening. One small, ventral, single genital sensillum or papilla (vs) on anterior cloacal lip. Papillae, cloacal opening, and phasmid (ph) arranged as (v1, v2d, v3/v4, ad, (ph, v5-v7, pd)); papilla nomenclature following Sudhaus & Fürst von Lieven (2003), whereby all ventral papillae and most anterior dorsal papilla are numbered by absolute position along body axis, dorsal papilla being appended with a 'd' (*i.e.*, v2d or v3d); remaining dorsal papillae being designated as anterior (ad) or posterior (pd). Papillae v1-ad of almost equal size, rather large and conspicuous, v5 and v6 relatively low and flattened, sometimes difficult to observe by LM, v7 and pd small but larger than v5 and v6, *i.e.*, intermediate between v1-ad and v5/v6 in size. Two small, flattened pairs, v5 and v6, each originating from a socket-like base; tip of v5 papillae split into two small papilla-like projections (laterally split into two tips), v6 with two small expansions, one anterior and one posterior, rendering papilla trifurcate. Morphology of v1-ad, v7, and pd papilliform and cone-shaped, originating directly in body wall. An extra pair of ventral papillae (v0) sometimes present *ca* one cloacal body diam. (CBD) anterior to v1. Tail *ca* two CBD long excluding terminal spike. Bursa or bursal flap absent. Tail conical, with short (less than one-third tail length) spike.

Female

Relaxed or slightly ventrally arcuate when killed by heat. Gonad didelphic, amphidelphic. Each genital system arranged from vulva and vagina as uterus, oviduct, and ovary. Anterior gonad to right of intestine, with uterus and oviduct extending ventrally and anteriorly on right of intestine and with a totally reflexed (= antidromous reflexion) ovary extending dorsally on right of intestine. Oocytes mostly arranged in multiple rows, sometimes more than five rows in distal third of ovary and in single row in remaining part, distal tips of each ovary reaching vulval region in well-fed mature females, although ovaries sometimes short and not reaching vulval region in young females or starved females from old cultures. Distal part of oviduct serving as spermatheca, mass of sperm sometimes observed in distal part of oviduct close to ovary. Eggs in single- to multiple-cell stage, or even further developed in proximal part of oviduct (= uterus). Distal part of oviduct (spermatheca) composed of relatively small

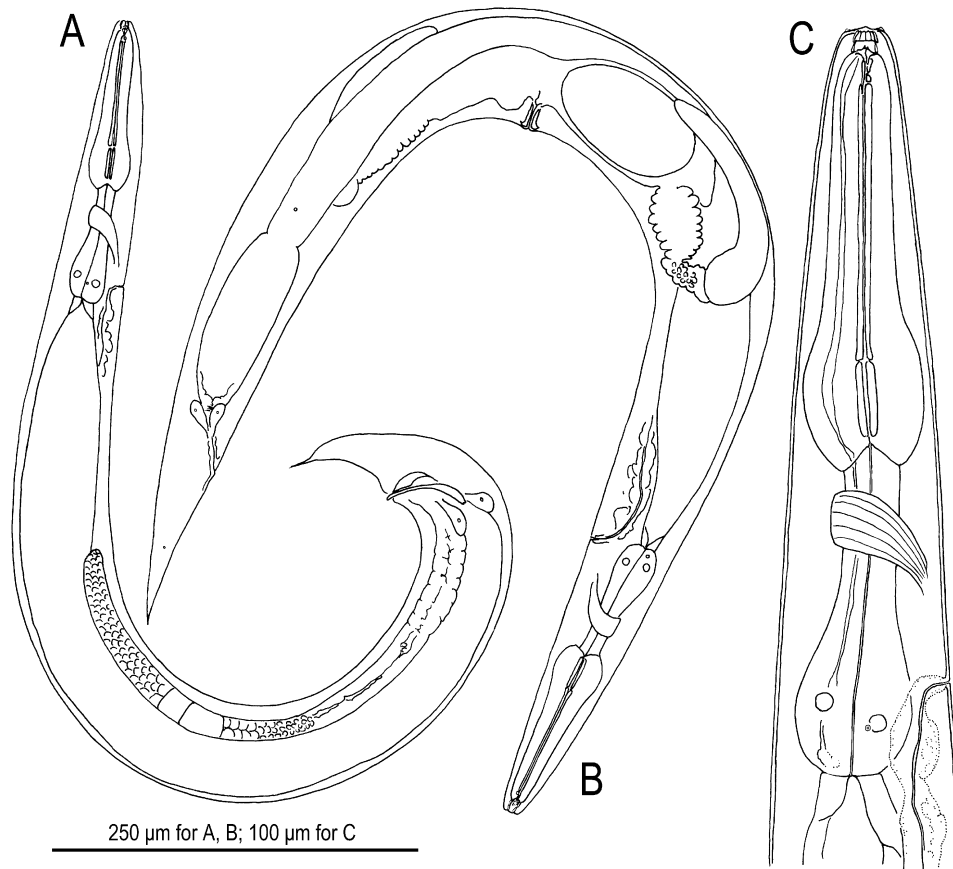


Fig. 2. *Allodiplogaster josephi* n. sp. A: Right lateral view of male; B: Right lateral view of female; C: Anterior region of female in right lateral view.

and rounded cells, middle part of oviduct (crustaformeria) composed of relatively large and rounded binuclear cells, presumably a shell gland. Distal part of oviduct composed of relatively large, flattened cells, connected to thick-walled uterus. *Receptaculum seminis* not observed. Dorsal wall of uterus at level of vulva thickened, appearing dark under LM. Four small vaginal glands present but obscure. Vagina perpendicular to body axis, surrounded by sclerotised tissue. Vulva slightly protuberant in lateral view, pore-like in ventral view. Rectum *ca* one anal body diam. (ABD) long, intestinal-rectal junction surrounded by well developed sphincter muscle. Three (two subventral and one dorsal) rectal (anal) glands present at intestinal/rectal junction but not obvious. Anus in form of dome-shaped slit, posterior anal lip slightly protuberant. Phasmid *ca* two ABD posterior to anus. Tail conical, without filiform extension.

DESCRIPTION OF SPECIES-SPECIFIC CHARACTERS

*Allodiplogaster josephi** n. sp.

= *Koerneria* sp. RGD227 *apud* Kanzaki *et al.*, 2009a, 2010a, 2011a, 2012a; Porazinska *et al.*, 2009, 2010a, b; Hazir *et al.*, 2010; Atighi *et al.*, 2013; Kanzaki & Giblin-Davis, 2014
 = *Koerneria* RG20 *apud* Fürst von Lieven, 2000; Fürst von Lieven & Sudhaus, 2000
 = *Allodiplogaster* sp. RGD227 *apud* Kanzaki *et al.*, 2014a
 (Figs 2-9)

MEASUREMENTS

See Table 1.

* Named in honour of Mr Joseph D.C. Giblin, first son of the second author.

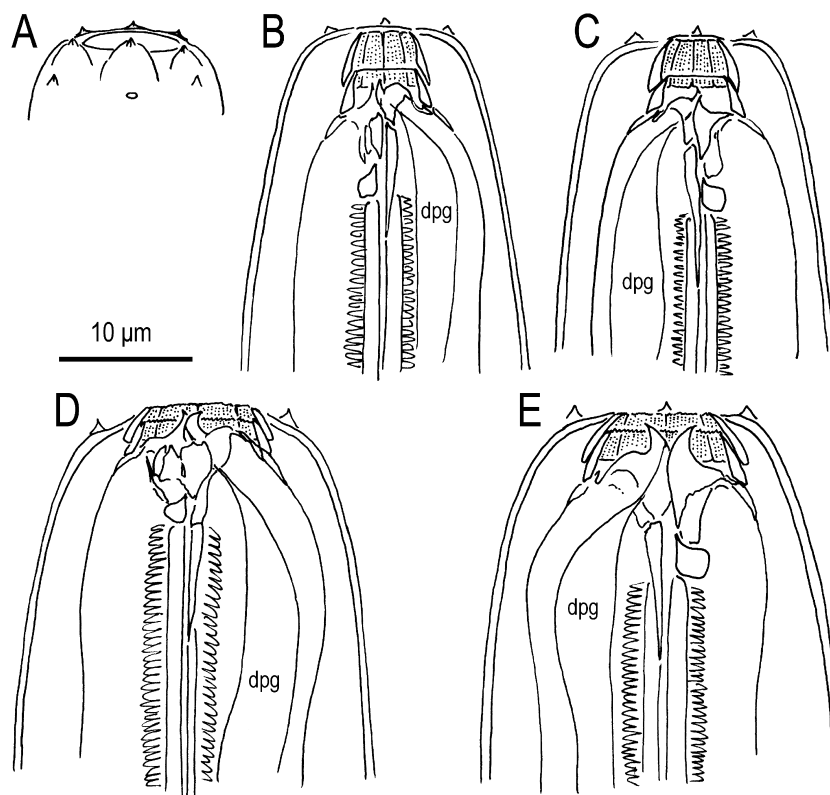


Fig. 3. Stomatal region of *Allodiplogaster josephi* n. sp. A: Lip region of adult stenostomatous male, showing labial sensilla, cephalic papillae and amphid; B: Left lateral view of stenostomatous form; C: Right lateral view of stenostomatous form; D: Left lateral view of eurystomatous form; E: Right lateral view of eurystomatous form. A-D: dpg = dorsal pharyngeal gland.

DESCRIPTION OF SPECIES-SPECIFIC CHARACTERS

Eurystomatous form

Found in both males and females in old (= starved) cultures. General morphology, except for left subventral sector of metastegostom, as described above; left subventral plate diagnostic of new species. Left subventral sector bearing three cuticular plates on ventral, subventral, and lateral sides. Lateral plate twice as large as other two plates, with two pointed, triangular projections at anterior end. Remaining two plates narrower than lateral plate, also with pointed anterior ends.

Male

Spicules slender, ventrally bent at 40% from anterior end. Manubrium squared, barely distinct from rest of spicule. Lamina-calomus complex (main part of spicule) smoothly tapering to bent position, with thickness consistent after bend of spicule posteriad to finely pointed and slightly ventrally curved tip, each tip with a ventral

hook visible only by SEM. Ventral expansion not observed in lamina-calomus. Distal thirds of each spicule fused, forming a narrow V shape in ventral view. Gubernaculum conspicuous, wide, semicircular in lateral view, *ca* half of spicule in length. Dorsal wall smoothly curved, with both anterior and posterior ends each slightly elongated and forming pointed tip. Ventral wall of anterior third dorsally concave. Posterior part of ventral wall with membrane-like extension overlapping spicule. Transparent, pouch-like tissue covering anterior half of gubernaculum. Nine pairs of genital papillae and single ventral sensillum present as described above. Positions of papillae: v1 *ca* 1.5 CBD anterior to cloacal opening (CO); v2d *ca* one-third CBD anterior to CO; v3 immediately anterior to CO; v4 *ca* one-third CBD posterior to CO; ad *ca* 1 CBD posterior to CO, and about the mid-point between CO and the root of tail spike; ph, v5-v7, and pd close to each other, together located slightly anterior to root of tail spike. Tail spike short, occupying less than 25% of total tail length.

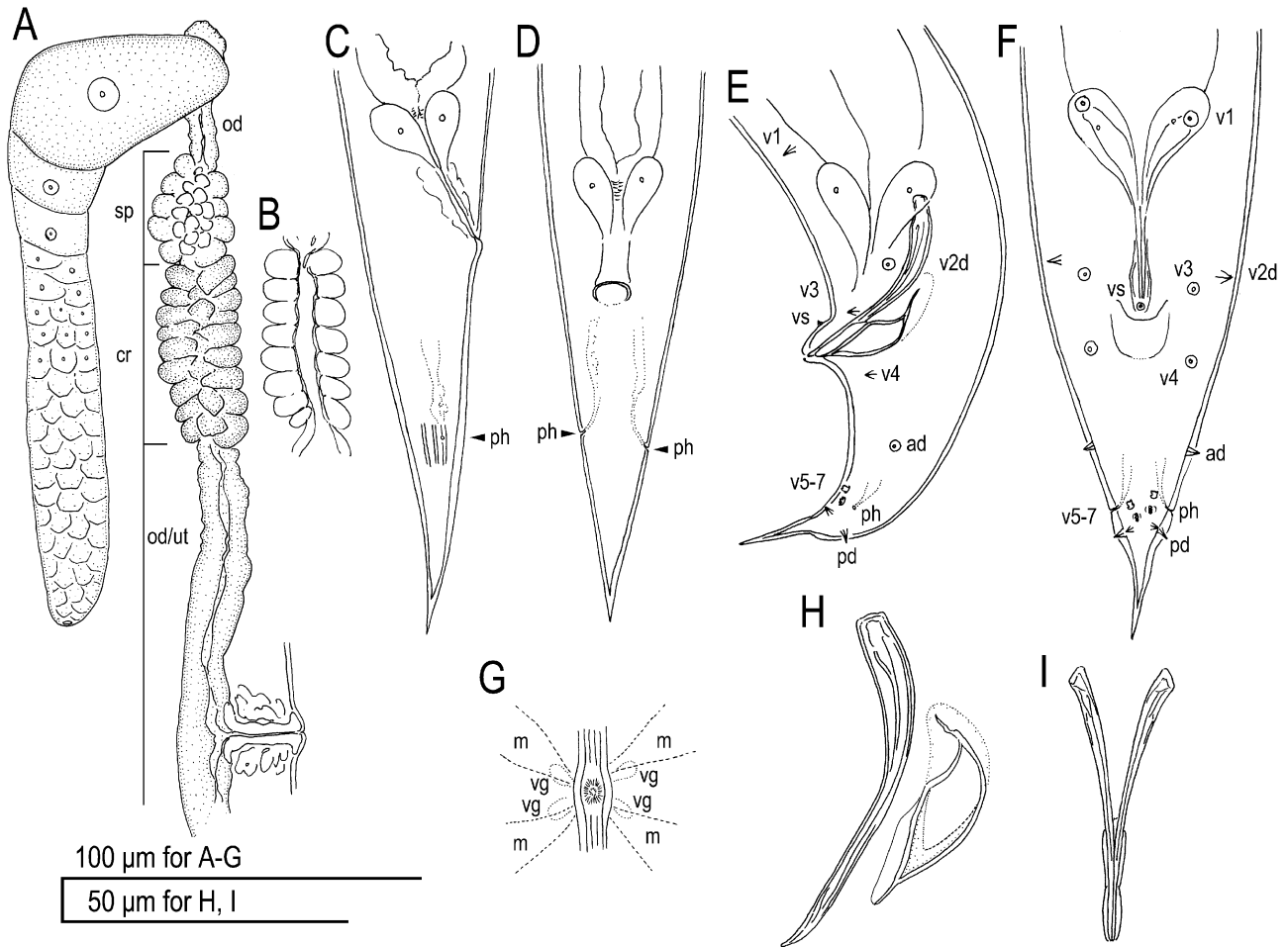


Fig. 4. *Allodiplogaster josephi* n. sp. A: Anterior gonad of female in right lateral view (od = oviduct; sp = spermatheca; cr = crustaformeria, or shell gland; od/ut = oviduct serving as uterus); B: Crustaformeria in different focal planes; C: Right lateral view of female tail (ph: phasmid); D: Ventral view of female tail (ph = phasmids); E: Left lateral view of male tail; F: Ventral view of male tail; G: Ventral view of vulval region (m = sphincter muscle; vg = vaginal gland); H: Left lateral view of spicule and gubernaculum; I: Ventral view of spicule and gubernaculum. Genital papillae and phasmid (ph) (E, F) labelled following nomenclature of Sudhaus & Fürst von Lieven (2003).

Female

Spermatheca composed of relatively small and rounded cells, easily distinguished from oviduct (tissue connecting spermatheca and ovary). Crustaformeria composed of rounded, glandular, binuclear cells, although nuclei not easily observed because of dark colour of cells under LM. Tail conical with pointed tip, less than 3 ABD long. Phasmid pore-like, *ca* 1.5 ABD posterior to anal opening, *i.e.*, located in posterior half of tail.

TYPE HOST AND LOCALITY

Allodiplogaster josephi n. sp. was cultured, as strain RGD227, from dauer nematodes present in the Dufour's gland of an adult female of *Colletes thoracicus* collected in Prince George's County, MD, USA, on 19 May 1986.

TYPE MATERIAL

Holotype stenostomatous male (USDANC collection number: T-684t), three paratype stenostomatous males (T-6482p-6484p), four paratype stenostomatous females

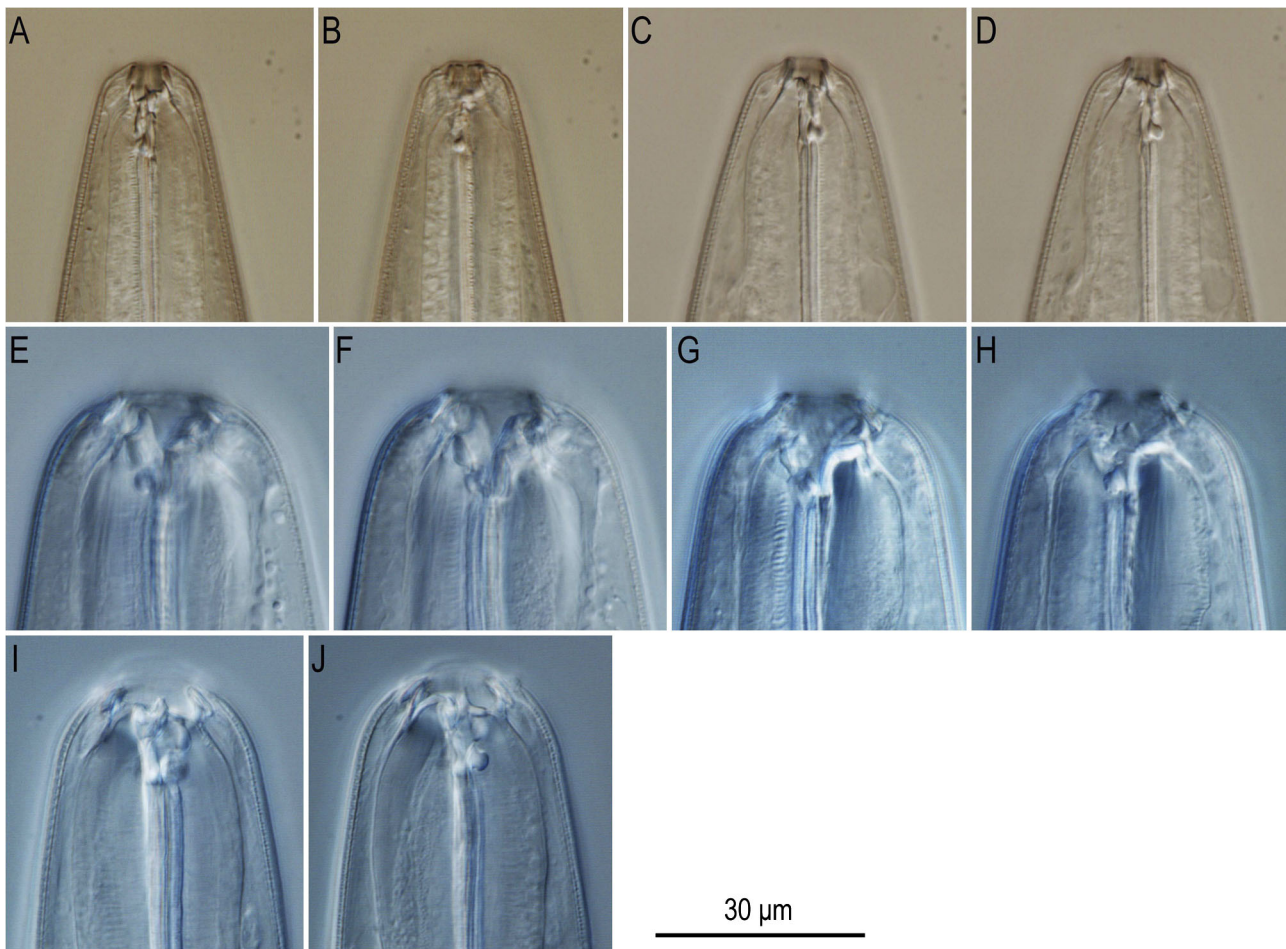


Fig. 5. Stomatal region of *Allodiplogaster josephi* n. sp. A, B: Left lateral view of stenostomatous female in different focal planes showing dorsal tooth (A) and left subventral denticles (B); C, D: Right lateral view of stenostomatous female in different focal planes showing dorsal tooth (C) and right subventral tooth (B); E-H: Left lateral view of eurystomatous form in different focal planes showing right subventral tooth and right subventral apodeme (E), dorsal tooth and ventral denticle plate (F), left subventral denticle plate (G), and left lateral denticle plate and left subventral apodeme (H); I, J: Right lateral view of eurystomatous form in different focal planes showing dorsal tooth (I) and right subventral tooth and apodeme (J). 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>.

(T-6485p-6488p), four paratype eurystomatous males (T-6489p-6492p), and four paratype eurystomatous females (T-6493p-6496p) deposited in the USDA Nematode Collection (USDANC), Beltsville, MD, USA. Four paratype stenostomatous males (UCRNC accession numbers 31422-31425), four paratype eurystomatous males (31426-31429), four paratype stenostomatous females (31430-31433), and four paratype eurystomatous females (31434-31436) deposited in the University of California Riverside Nematode Collection (UCRNC), Riverside, CA, USA. Two paratype stenostomatous males (FFPRI

collection number: *Allodiplogaster josephi* PM01-02), two paratype stenostomatous females (*Allodiplogaster josephi* PF01-02), two paratype eurystomatous males (*Allodiplogaster josephi* PM03-04), and two paratype eurystomatous females (*Allodiplogaster josephi* PF03-04) deposited in the Forest Pathology Laboratory Collection, Forestry and Forest Products Research Institute, Tsukuba, Japan. In addition to the type material, several voucher specimens from the type locality are deposited at the Fort Lauderdale Research and Education Center, University of Florida, Fort Lauderdale, FL, USA. The type strain of *A.*

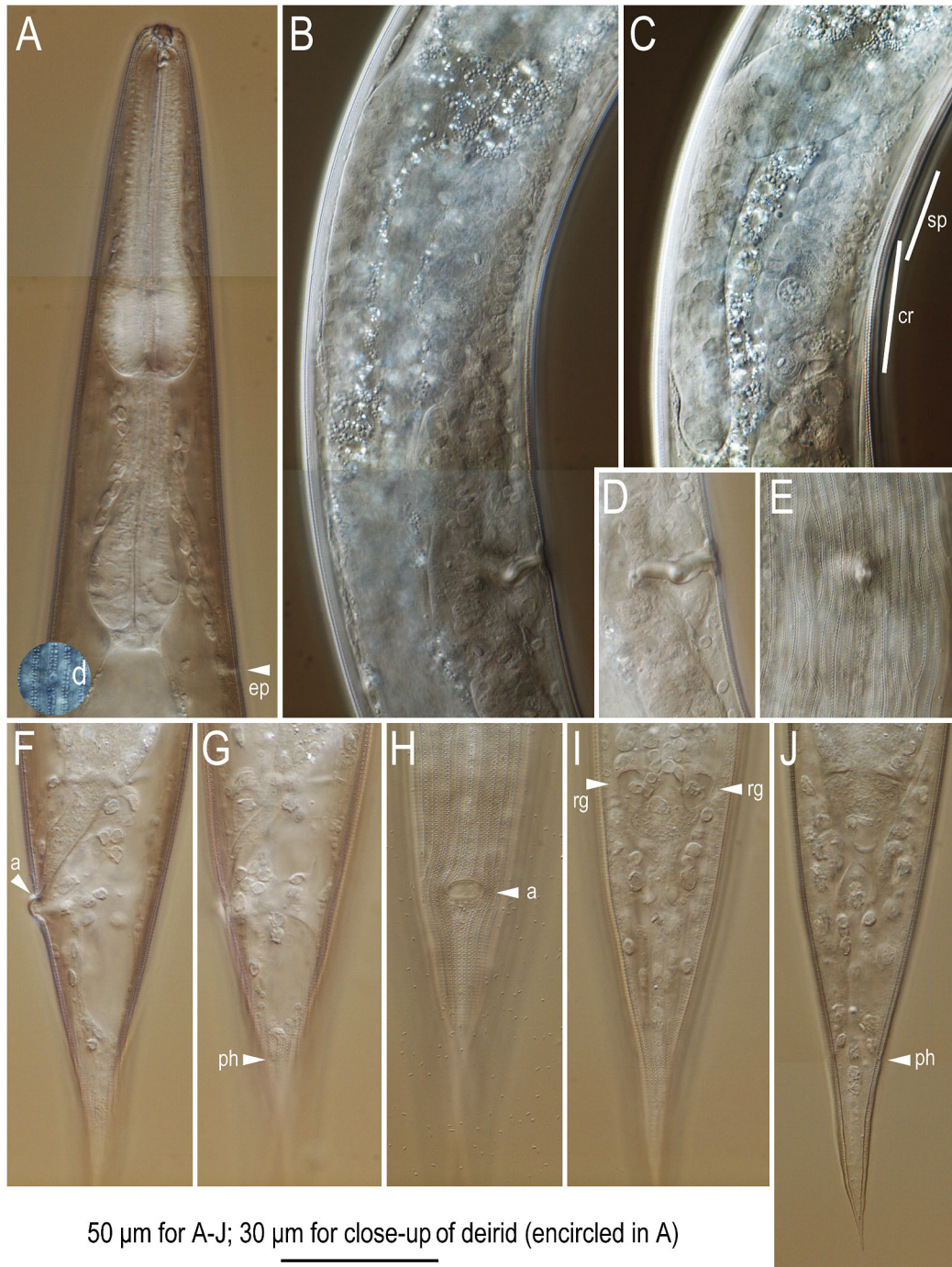


Fig. 6. *Allodiplogaster josephi* n. sp. A: Right lateral view of female adult (d: deirid is encircled at the original level; ep: excretory pore opening); B: Right lateral view of anterior female gonad; C: Different focal plane of (B) (sp: spermatheca; cr: crustaformeria, or shell gland); D: Different focal plane of (B) showing vulva and vagina; E: Ventral view of vulval opening; F, G: Left lateral view of female tail showing rectum and anus (F) and phasmid (G) (a: anus; ph: phasmid); F-J: Ventral view of female tail in different focal planes, showing anus (H), rectal (anal) gland (I) and phasmid (J) (a: anus; rg: rectal gland; ph: phasmid). 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>.

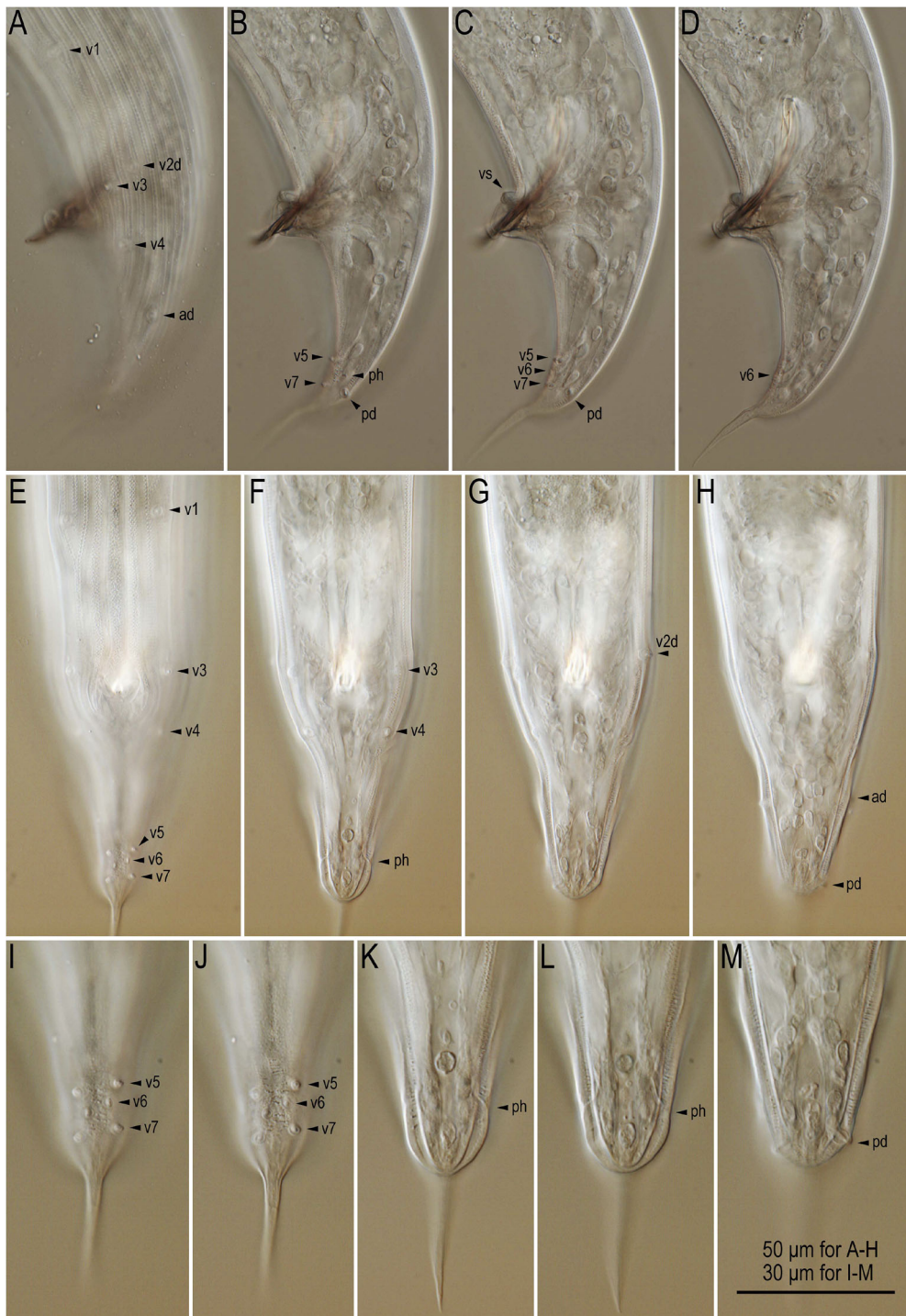


Fig. 7. Male tail region of *Allodiplogaster josephi* n. sp. A-D: Left lateral view in different focal planes; E-H: Ventral view in different focal planes; I-M: Ventral view of tail tip region in different focal planes. Genital papillae (v + number) and phasmid (ph) (E, F) are labelled following nomenclature of Sudhaus & Fürst von Lieven (2003). 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>.

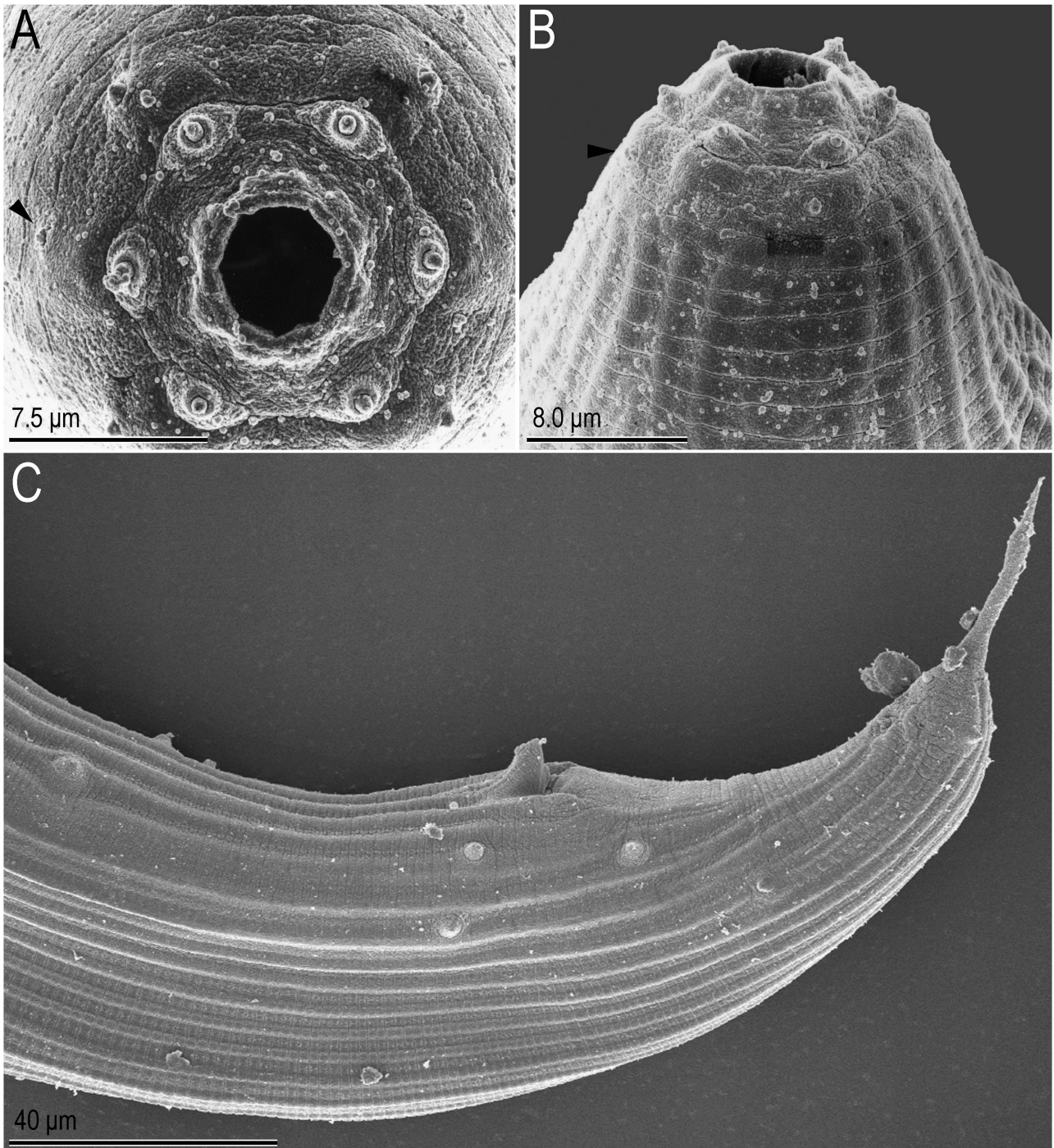


Fig. 8. Scanning electron micrographs of a stenostomatous male of *Allodiplogaster josephi* n. sp. A: En face view; B: Oblique dorsal view of head; C: Lateral view of tail region. Arrowheads indicate amphidial apertures (A, B).

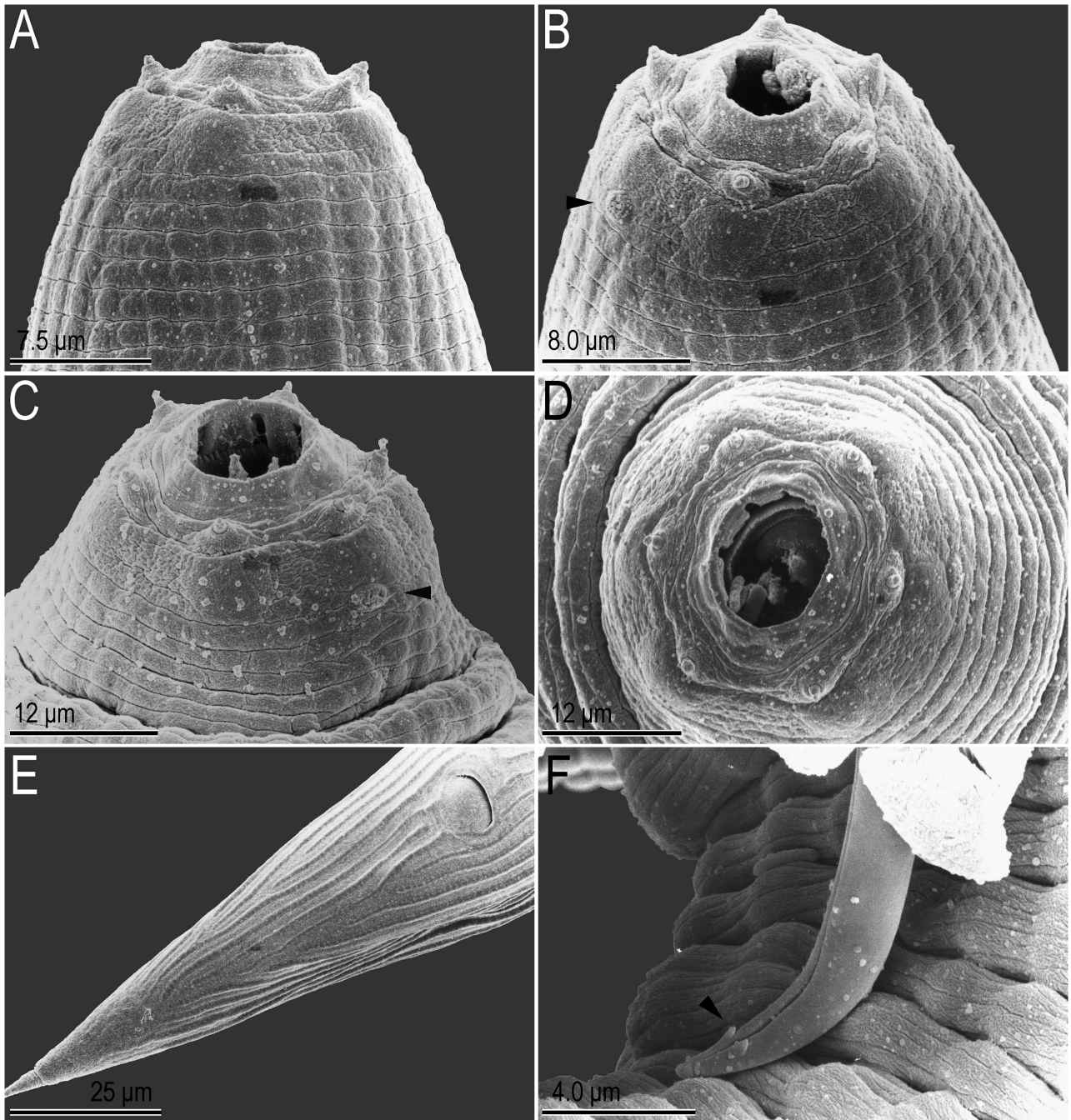


Fig. 9. Scanning electron micrographs of females and a male of *Allodiplogaster josephi* n. sp. A: Oblique dorsal view of head of female stenostomatous form; B: Oblique dorsal face view of stenostomatous female; C: Near right lateral view of head of eurystomatous female; D: *En face* view of eurystomatous female; E: Ventral view of female tail; F: Lateral view of male spicule tips. Arrowheads indicate amphidial apertures (B, C) or ventral hook (F).

josephi n. sp. (RGD227) is available as a living culture at Indiana University (EJR) and can be provided to other researchers upon request.

***Allodiplogaster seani** n. sp.**

= *Koerneria* sp. RS1982 *apud* Mayer *et al.*, 2007, 2009; Kanzaki *et al.*, 2012b, c, 2013a, b, c, 2014b, c; Herrmann *et al.*, 2013; Ragsdale *et al.*, 2013a, 2014; Susoy *et al.*, 2013

= *Koerneria* sp. RGD228 *apud* Kanzaki *et al.*, 2009a, 2010a, c, 2011a, 2012a, 2013d, 2014d;

Porazinska *et al.*, 2009, 2010a, b;

Hazir *et al.*, 2010; Atighi *et al.*, 2013

= *Allodiplogaster* sp. RGD228 *apud*

Kanzaki *et al.*, 2014a

= *Allodiplogaster* sp. RS1982 *apud*

Kanzaki *et al.*, 2014a; Susoy *et al.*, 2015

(Figs 10-18)

MEASUREMENTS

See Table 2.

DESCRIPTION OF SPECIES-SPECIFIC CHARACTERS

Eurystomatous form

Found in both males and females in old (= starved) cultures. General morphology, except for left subventral sector of metastegostom, as described above, left subventral plate diagnostic of new species. Left subventral sector bearing three cuticular plates on ventral, subventral and lateral sides. Lateral plate twice as large as other two plates, with one large triangular projection at anterior end. Remaining two plates narrower than lateral plate, also with pointed anterior ends.

Male

Spicules slender, weakly ventrally bent at 40% from anterior end. Manubrium a rounded quadrilateral, barely distinct from rest of spicule. Lamina-calomus complex (main part of spicule) smoothly narrowing to bent position, with thickness consistent after bend in spicule posteriorly to finely pointed and weakly ventrally curved tip; each tip with a ventral hook visible only by SEM. Ventral

expansion not clearly observed in lamina-calomus. In ventral aspect, anterior half of spicule clearly separated forming a Y shape. Gubernaculum conspicuous, wide, semi-circular in lateral view, *ca* half of spicule in length. Dorsal wall smoothly curved, anterior end slightly elongated and forming pointed tip. Ventral wall of anterior 40% of gubernaculum dorsally concave. Posterior part of ventral wall with membrane-like extension overlapping spicules. Transparent, pouch-like tissue covering anterior half of gubernaculum. Nine pairs of genital papillae and single ventral sensillum or papilla present as described above. Positions of papillae: v1 *ca* 1.5 CBD anterior to CO; v2d *ca* one-third CBD anterior to CO; v3 immediately anterior to CO; v4 *ca* one-third CBD posterior to CO; ad *ca* 1 CBD posterior to CO and at about mid-point between CO and root of tail spike; ph, v5-v7, and pd close to each other, together located slightly anterior to root of tail spike. Extra pair of papillae (v0) present in some individuals at 0.5-1.0 CBD anterior to v1. Tail spike short, occupying less than one-third of total tail length.

Female

Spermatheca composed of rounded cells, weakly distinguished from oviduct (tissue connecting spermatheca and ovary). Crustaformeria composed of large, rounded, glandular, and binuclear cells, appearing dark under LM, with nuclei easily observed in some individuals. Junction between crustaformeria and uterus with wrinkle-like pattern. Tail conical to slightly elongate conical, with pointed tip. Tail length varying among individuals. Phasmid pore-like, *ca* two ABD posterior to anal opening, *i.e.*, located in anterior half of tail.

TYPE HOST AND LOCALITY

Allodiplogaster seani n. sp. was cultured, as strain RGD228, from dauer nematodes isolated from the abdominal glands of a female of *Andrena alleghaniensis* collected on 20 June 1987 from the central Adirondack Mountains near Saranac Lake, Franklin County, NY, USA.

OTHER HOST AND LOCALITY

In addition to the type host and locality, *A. seani* n. sp. was previously collected from the May beetle, *Melolontha melolontha* Fabricius (Coleoptera: Scarabaeidae), in Usedom, Germany (Mayer *et al.*, 2007).

* Named in honour of Mr Sean M. Giblin, second son of the second author and whose perseverance in the face of great hardship has touched and inspired many.

Table 1. Morphometrics of *Allodiplogaster josephi* n. sp. (RGD227). All measurements are in μm and in the form: mean \pm s.d. (range).

Character	Stenostomatous form			Eurystomatous form	
	Male		Female	Male	Female
	Holotype	Paratypes	Paratypes	Paratypes	Paratypes
n	–	9	10	10	10
L	1173	1171 \pm 72 (1008-1254)	1468 \pm 136 (1235-1694)	819 \pm 49 (759-987)	1021 \pm 90 (924-1224)
a	18.3	18.9 \pm 1.7 (16.7-22.0)	15.8 \pm 1.1 (13.8-17.6)	18.6 \pm 1.3 (16.0-20.1)	16.7 \pm 1.8 (13.1-19.0)
b	6.0	5.9 \pm 0.3 (5.2-6.4)	6.8 \pm 0.5 (5.9-7.5)	1.5 \pm 0.2 (4.2-4.6)	4.9 \pm 0.2 (4.7-5.5)
c	12.6	13.5 \pm 0.8 (12.2-14.8)	12.2 \pm 0.6 (11.3-13.3)	10.5 \pm 1.0 (9.2-11.9)	10.3 \pm 0.4 (9.8-11.2)
c'	2.1	2.1 \pm 0.1 (2.0-2.3)	3.3 \pm 0.4 (2.8-4.1)	2.2 \pm 0.2 (2.0-2.6)	3.2 \pm 0.3 (2.5-3.6)
T or V	63.9	63.7 \pm 4.4 (56.9-70.4)	53.4 \pm 1.2 (51.4-54.9)	47.5 \pm 2.8 (42.0-51.3)	54.4 \pm 0.7 (53.4-55.6)
Lip diam.	10.5	11.0 \pm 0.5 (9.9-11.7)	11.7 \pm 0.7 (10.5-12.9)	11.7 \pm 0.7 (10.5-12.3)	15.4 \pm 1.0 (12.9-17.0)
Stoma width (opening)	3.5	3.4 \pm 0.5 (2.9-4.1)	3.7 \pm 0.4 (2.9-4.1)	5.0 \pm 0.3 (4.7-5.3)	7.2 \pm 0.7 (6.4-8.8)
Stoma width (widest part)	5.8	5.1 \pm 0.6 (4.1-5.8)	5.4 \pm 0.6 (4.7-6.4)	7.5 \pm 0.7 (6.4-8.2)	10.8 \pm 0.5 (9.9-11.7)
Stoma depth	13.5	13.2 \pm 0.6 (12.3-14.6)	13.7 \pm 0.5 (13.5-14.6)	13.0 \pm 0.3 (12.9-13.5)	14.3 \pm 0.8 (13.5-15.2)
Anterior pharynx length	104	107 \pm 3.6 (104-113)	120 \pm 2.2 (116-122)	102 \pm 4.6 (92-108)	116 \pm 5.7 (106-124)
Widest part of median bulb from anterior	99	99 \pm 4.3 (89-106)	108 \pm 3.8 (99-113)	93 \pm 4.1 (87-99)	107 \pm 5.8 (97-117)
Median bulb diam.	29	28 \pm 1.2 (26-30)	33 \pm 1.4 (31-36)	27 \pm 0.7 (25-27)	30 \pm 3.0 (26-34)
Posterior pharynx length	85	83 \pm 3.4 (78-90)	91 \pm 3.2 (87-96)	75 \pm 3.9 (66-79)	83 \pm 7.7 (70-91)
Nerve ring from anterior	135	134 \pm 4.2 (128-140)	147 \pm 3.3 (140-151)	121 \pm 5.5 (113-130)	138 \pm 7.0 (128-148)
Widest part of basal bulb from anterior	177	180 \pm 6.9 (170-192)	200 \pm 4.4 (194-206)	168 \pm 8.0 (155-180)	187 \pm 11.2 (170-203)
Basal bulb diam.	25	26 \pm 1.5 (22-27)	30 \pm 1.5 (28-33)	24 \pm 0.7 (22-24)	28 \pm 3.1 (24-32)
Excretory pore from anterior	190	183 \pm 13 (152-197)	201 \pm 13 (178-219)	143 \pm 7.1 (133-153)	163 \pm 9.9 (147-179)
Anterior/posterior pharynx ratio	1.22	1.29 \pm 0.1 (1.22-1.36)	1.31 \pm 0.1 (1.26-1.39)	1.37 \pm 0.1 (1.30-1.48)	1.39 \pm 0.1 (1.31-1.58)
Max. body diam.	64	62 \pm 6.8 (50-70)	93 \pm 12.7 (79-122)	44 \pm 5.1 (38-54)	62 \pm 11.8 (51-81)
Testis length	750	748 \pm 89 (573-882)	–	389 \pm 35 (339-434)	–
Anterior ovary length	–	–	270 \pm 65 (154-350)	–	189 \pm 31 (165-264)
Posterior ovary length	–	–	277 \pm 75 (118-357)	–	166 \pm 27 (123-207)

Table 1. (Continued.)

Character	Stenostomatous form			Eurystomatous form	
	Male		Female	Male	Female
	Holotype	Paratypes	Paratypes	Paratypes	Paratypes
Female rectum length	–	–	42 ± 2.5 (38-46)	–	34 ± 3.7 (27-41)
Cloacal or anal body diam.	44	42 ± 1.1 (40-44)	37 ± 3.0 (32-42)	35 ± 2.1 (31-38)	32 ± 3.8 (26-38)
Tail length including tail spike	93	87 ± 4.0 (82-93)	120 ± 8.0 (110-135)	78 ± 6.2 (69-85)	99 ± 6.5 (89-110)
Tail spike length	36	27 ± 3.9 (21-36)	–	24 ± 1.7 (20-26)	–
Spicule length (line)	62	61 ± 1.6 (59-64)	–	55 ± 1.8 (52-57)	–
Spicule length (curve)	75	70 ± 4.2 (63-77)	–	61 ± 1.9 (58-63)	–
Gubernaculum length (line)	41	39 ± 1.9 (36-42)	–	35 ± 2.3 (31-39)	–
Female phasmid (distance from anus)	–	–	54 ± 4.1 (47-60)	–	46 ± 5.1 (40-56)
Female phasmid position (relative position to tail length in %)	–	– (40.3-49.6)	45.1 ± 3.1	–	46.5 ± 4.4 (37.3-52.6)

TYPE MATERIAL

Holotype stenostomatous male (USDANC collection number: T-685t), three paratype stenostomatous males (T-6497p-6499p), four paratype stenostomatous females (T-6500p-6503p), and four paratype eurystomatous females (T-6504p-6507p) deposited in the USDA Nematode Collection (USDANC), Beltsville, MD, USA. Four paratype stenostomatous males (31438-31441), four paratype stenostomatous females (31442-31445), and four paratype eurystomatous females (31446-31449) deposited in the University of California Riverside Nematode Collection (UCRNC), Riverside, CA, USA. Two paratype stenostomatous males (FFPRI collection number: *Allodiplogaster seani* PM01-02), two paratype stenostomatous females (*Allodiplogaster seani* PF01-02), and two paratype eurystomatous females (*Allodiplogaster seani* PF03-04) deposited in the Forest Pathology Laboratory Collection, Forestry and Forest Products Research Institute, Tsukuba, Japan. In addition to the type material, several voucher specimens from the type locality are deposited at the Fort Lauderdale Research and Education Center, University of Florida, Fort Lauderdale, FL, USA. Both strains of *A. seani* n. sp. (RGD228, RS1982) are available as living cultures and as frozen stocks at Indiana

University (EJR) and can be provided to other researchers upon request.

DIAGNOSIS AND RELATIONSHIPS OF THE TWO NEW SPECIES

In addition to characters common to both new species, *A. josephi* n. sp. is diagnosed by the spicules, each of which is clearly bent at 40% from the anterior end and has an indistinct squared manubrium, and the short and conical female tail. The nature of the host association, whereby nematodes are carried as dauers in the Dufour's glands of female *Colletes* bees, is also specific to *A. josephi* n. sp. The high induction of the eurystomatous morph in both sexes, as compared with *A. seani* n. sp., may be diagnostic of the species, although the prevalence of stomatal morphs are known to differ also within a single species (Ragsdale *et al.*, 2013b).

Allodiplogaster seani n. sp. is characterised by the spicule being weakly bent at 40% of the distance from the anterior end and with a barely distinct, rounded quadrilateral-shaped manubrium and relatively short and conical female tail. The nature of the host association, whereby nematodes are carried as dauers in the abdominal

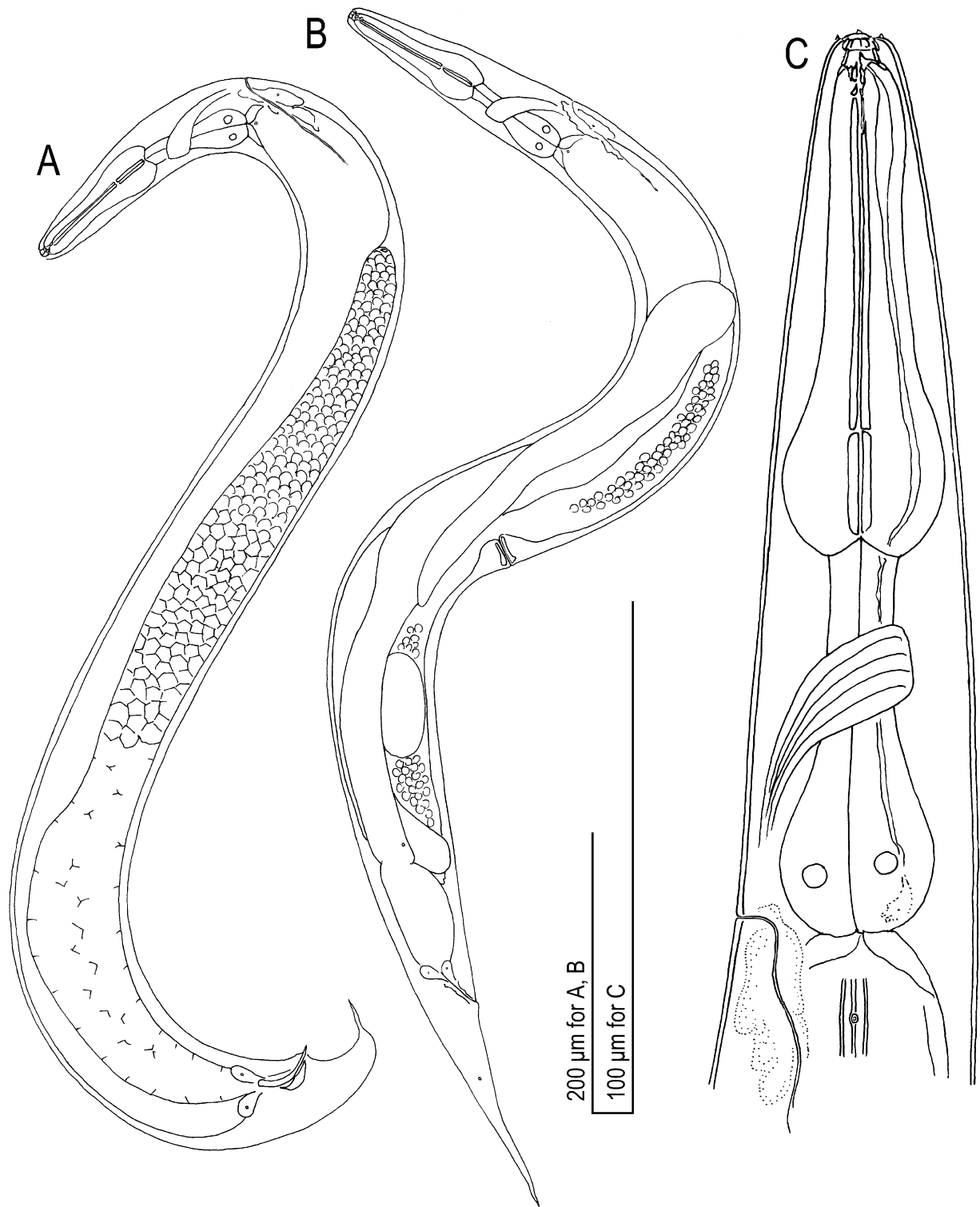


Fig. 10. *Allodiplogaster seani* n. sp. A: Right lateral view of male; B: Right lateral view of female; C: Anterior region of female in left lateral view.

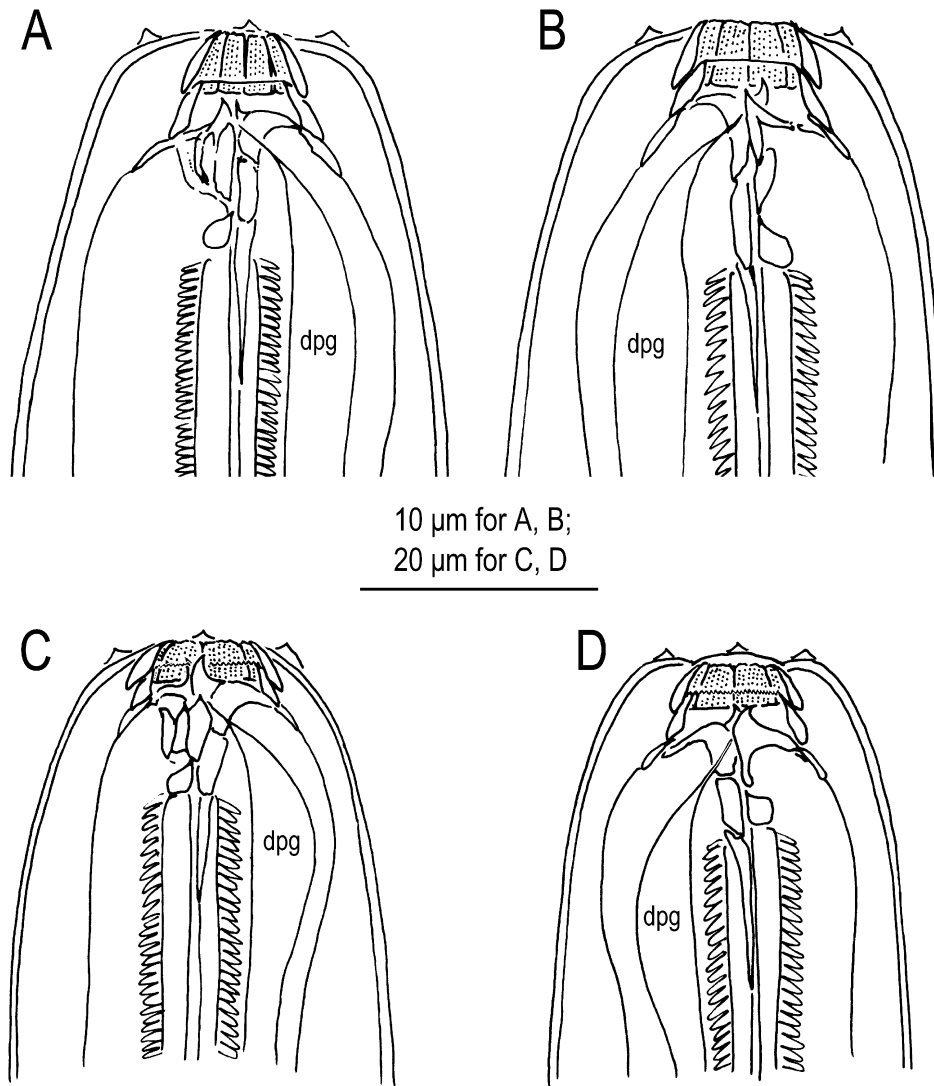


Fig. 11. Stomatal region of *Allodiplogaster seani* n. sp. A: Left lateral view of stenostomatous form; B: Right lateral view of stenostomatous form; C: Left lateral view of eurystomatous form; D: Right lateral view of eurystomatous form. B-E: dpg = dorsal pharyngeal gland.

glands of female *Andrena* bees, is also a potentially informative character of the species.

The morphology of the female reproductive tract may be reliable for diagnostics, although this morphology has not been comparably described for most other species of *Allodiplogaster*. Thus, the character is excluded from comparisons herein.

The genus *Allodiplogaster* Paramonov & Sobolev in Skrjabin, Shikobalova, Sobolev, Paramonov & Sudarikov, 1954 had been a junior synonym of *Koerneria* Meyl, 1960, but was recently resurrected (Kanzaki *et al.*, 2014a).

Allodiplogaster was further separated into two groups: i) the *henrichae* group, containing 13 terrestrial insect associates; and ii) the *striata* group, containing 19 aquatic species.

Both new species belong to the *henrichae* group, which includes *A. henrichae* (Sachs, 1950) Paramonov & Sobolev in Skrjabin, Shikobalova, Sobolev, Paramonov & Sudarikov, 1954 (type species), *A. colobocerca* (Andrássy, 1964) Kanzaki, Ragsdale & Giblin-Davis, 2014a, *A. hirschmannae* (Sachs, 1950) Kanzaki, Ragsdale & Giblin-Davis, 2014a, *A. histophora* (Weingärtner, 1955) Kan-

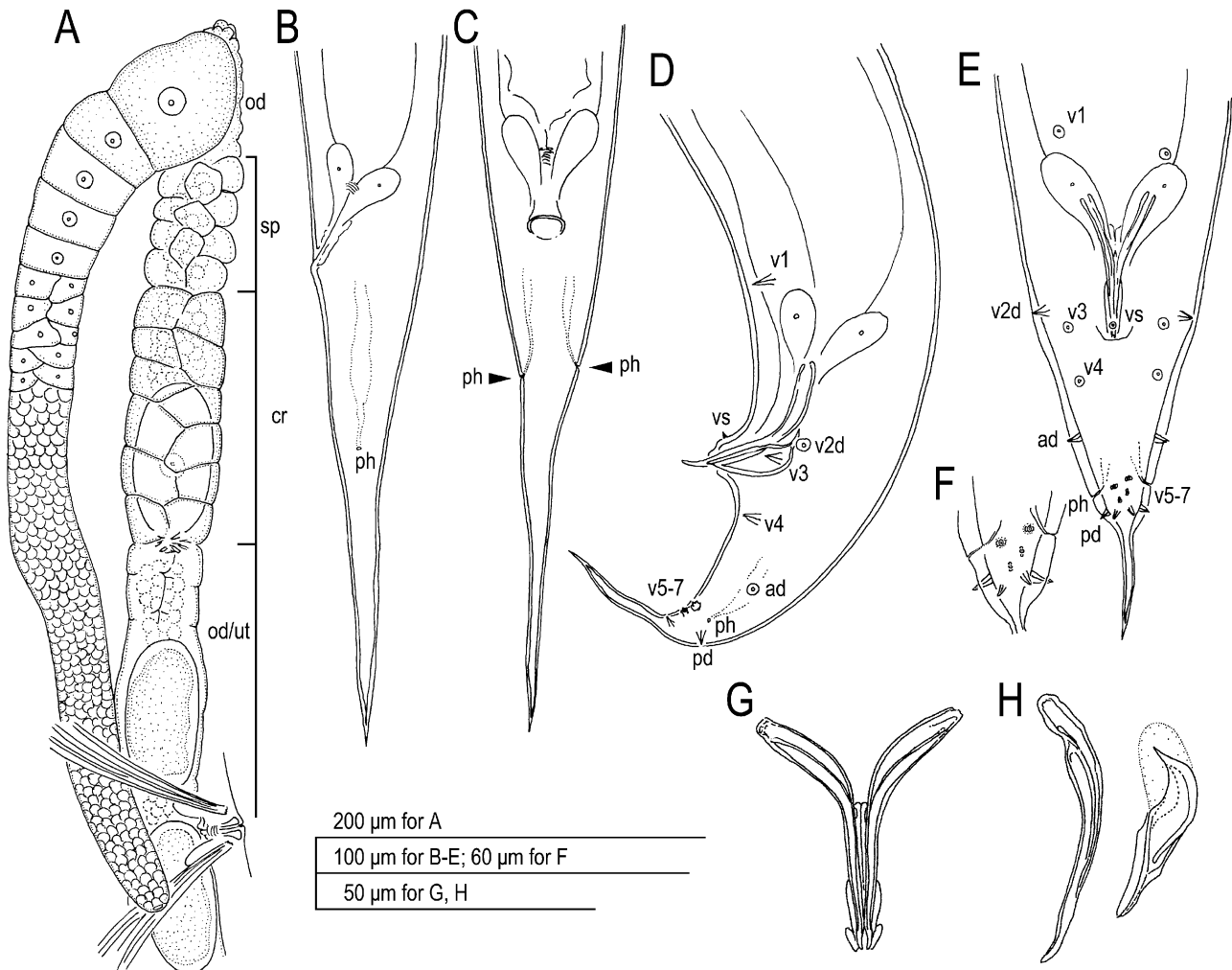


Fig. 12. *Allodiplogaster seani* n. sp. A: Anterior gonad of female in the right lateral view (od = oviduct; sp = spermatheca; cr = crustaformeria, or shell gland; od/ut = oviduct serving as uterus); B: Right lateral view of female tail (ph = phasmid); C: Ventral view of female tail (ph = phasmid); D: Left lateral view of male tail; E: Ventral view of male tail; F: Ventral view of posterior part of male tail; G: Ventral view of spicule and gubernaculum; H: Left lateral view of spicule and gubernaculum. Genital papillae and phasmid (ph) (E, F) labelled following nomenclature of Sudhaus & Fürst von Lieven (2003).

zaki, Ragsdale & Giblin-Davis, 2014a, *A. hylobii* (Fuchs, 1915) Kanzaki, Ragsdale & Giblin-Davis, 2014a, *A. incurva* (Körner, 1954) Kanzaki, Ragsdale & Giblin-Davis, 2014a, *A. labiomorpha* (Kühne, 1995) Kanzaki, Ragsdale & Giblin-Davis, 2014a, *A. lepida* (Andrássy, 1958) Kanzaki, Ragsdale & Giblin-Davis, 2014a, *A. lucani*, *A. pierci* (Massey, 1967) Kanzaki, Ragsdale & Giblin-Davis, 2014a, *A. pini* (Fuchs, 1931) Kanzaki, Ragsdale & Giblin-Davis, 2014a, *A. robinicola* (Rühm, 1956) Kanzaki, Ragsdale & Giblin-Davis, 2014a, and *A. sudhausi* (Fürst von Lieven, 2008) Kanzaki, Ragsdale & Giblin-Davis, 2014a.

Based on morphological characters, both *A. josephi* n. sp. and *A. seani* n. sp. are close to *A. henrichae*, *A. histophora*, *A. labiomorpha*, *A. lucani*, *A. pierci*, *A. pini* and *A. robinicola*. These seven species share the features of a relatively short male tail and conical female tail. However, *A. josephi* n. sp. is distinguished from these other species as follows: from *A. henrichae* by the position of the v1 papillae, which is ca 1.2 vs 2 CBD anterior to CO, by spicule morphology (distinct manubrium absent vs present), by gubernaculum morphology (anterior 35% vs 20% of ventral wall dorsally concave), the length of

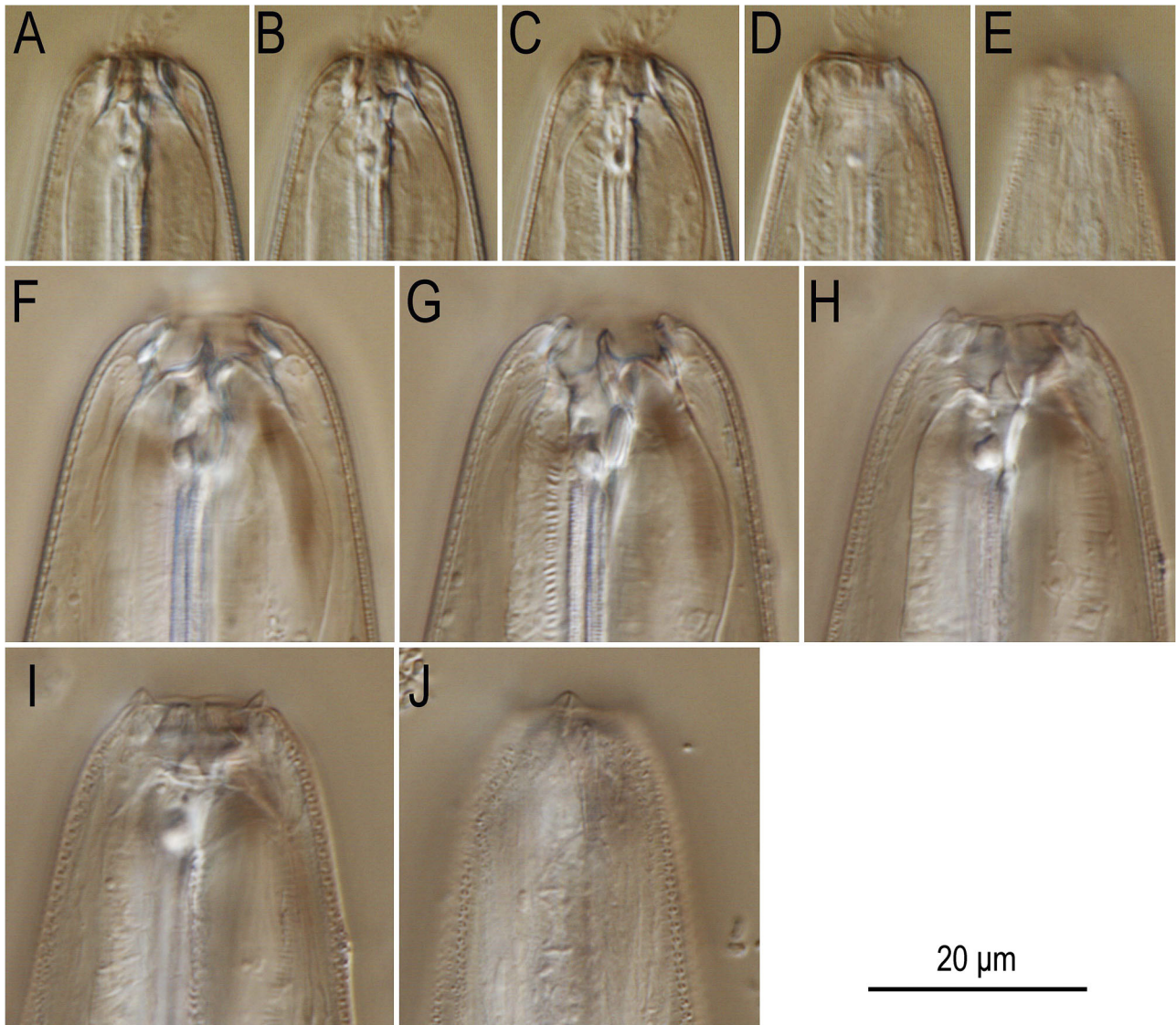


Fig. 13. Left lateral view of stomatal region of *Allodiplogaster seani* n. sp. A-E: Stenostomatous male in different focal planes showing right subventral tooth and apodeme (A), dorsal tooth and ventral denticle (B), dorsal tooth and left subventral and left lateral denticles (C), pro- + mesostegostom and left subventral apodeme (D) and cephalic papilla and amphid (E); F-J: Eurystomatous female in different focal planes, showing right subventral tooth, apodeme, and ventral denticle plate (F), dorsal tooth and left subventral denticle plate (G), dorsal tooth, left lateral denticle plate and left subventral apodeme (H), pro- + mesostegostom and chielostomatal plates (I), and amphid (J). 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 male tail spike (less than 25% vs more than 33% of the total tail length), and by the female tail (short and conical ($c' = 3.3$ (2.8-4.1) in stenostomatous form and 3.2 (2.5-3.6) in eurystomatous form) vs elongate conical ($c' = 4.3$, 5.4 as calculated from the drawings in original description and redescription, respectively));

from *A. histophora* by the position of the male phasmid (close to v5-7 and pd vs close to ad), and by the female tail (short conical vs a slightly elongated conical) (Weingärtner, 1955; Kühne, 1995); from *A. labiomorpha* by gubernaculum morphology (anterior 33 vs 20% of its ventral wall being dorsally concave), and a female tail

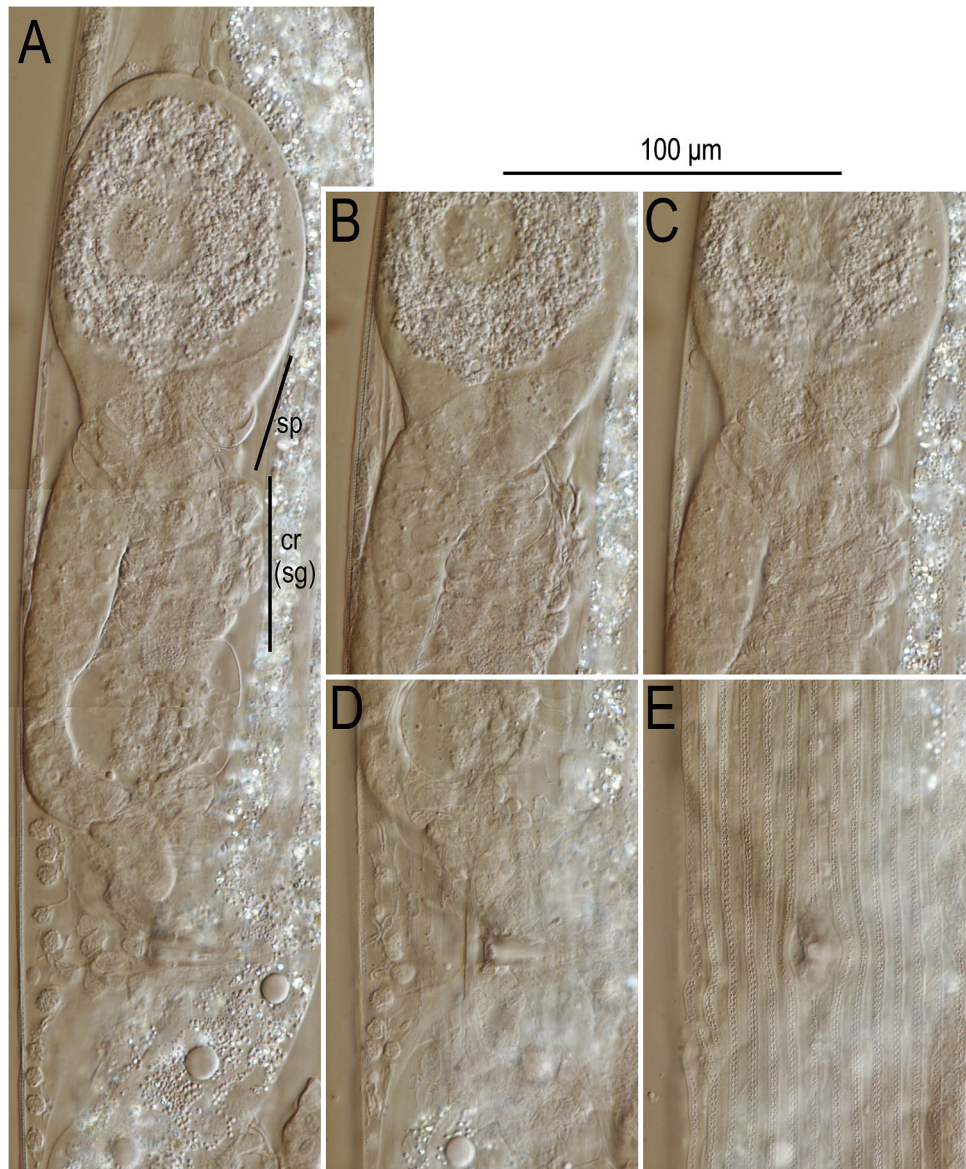


Fig. 14. Ventral view of anterior female gonad of *Allodiplogaster seani* n. sp. A: Entire gonad; B, C: Different focal planes of spermatheca and crustaformeria; D, E: Different focal planes of vulval region. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/15685411>.

that is short and conical ($c' = 3.3$ (2.8-4.1); see above) vs elongated conical ($c' = 5.9$ as calculated from the drawing in original description) (Kühne, 1995); from *A. lucani* by the male spicule being clearly bent with an indistinctive squared manubrium vs thin and smoothly arcuate with a rounded manubrium, gubernaculum wide vs thin, and by a female tail that is short and conical ($c' = 3.3$ (2.8-4.1); see above) vs elongate conical ($c' = 5.0$ as calculated

from the drawing in the original description); from *A. pierci* by its spicules, which are each clearly bent with an indistinctive, squared manubrium vs thin and smoothly arcuate with a rounded manubrium, gubernaculum wide vs thin, male tail spike less than 25% vs more than 33% of total tail length, and by a female tail that is short and conical ($c' = 3.3$ (2.8-4.1); see above) vs elongate conical ($c' = 5.9$ as calculated from the drawing in original

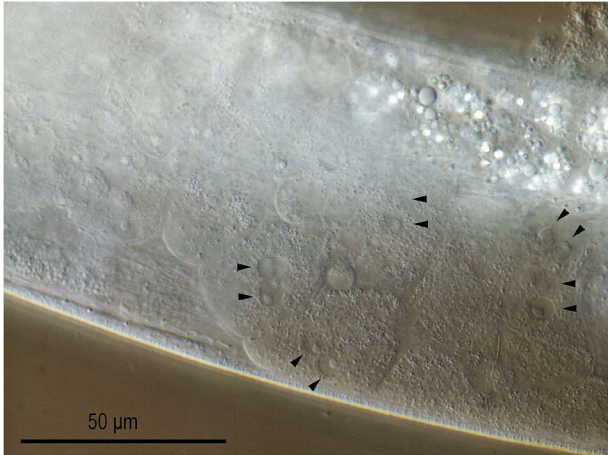


Fig. 15. Female crustaformeria (shell gland) of *Allodiplogaster seani* n. sp. Each shell gland cell has two nuclei (arrowheads). This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/15685411>.

description); from *A. pini* by its spicules, which are each clearly bent with an indistinctive squared manubrium *vs* thin and smoothly arcuate with a rounded manubrium and by a gubernaculum that is wide *vs* thin (Fuchs, 1931); from *A. robinicola* by the length of the male tail spike, which is less than 25% of *vs* ca 50% of total tail length, by the shape of the female tail, which is short and conical ($c' = 3.3$ (2.8-4.1); see above) *vs* elongate ($c' = 6.1$ as calculated from the drawing in original description), and by spicule form (distinct manubrium with a clear constriction absent *vs* present).

Allodiplogaster seani n. sp. is distinguished from *A. henrichae* by the position of the v1 papillae, ca 1.2 *vs* 2 CBD anterior to CO, by spicule morphology (distinct manubrium absent *vs* present), gubernaculum morphology (anterior 40 *vs* 20% of ventral wall dorsally concave); from *A. histophora* by the position of the male phasmid, which is close to v5-7 and pd *vs* close to ad; from *A. labiomorpha* by gubernaculum morphology (anterior 40 *vs* 20% of ventral wall dorsally concave) (Kühne, 1995); from *A. lucani* by spicules that are slightly bent and with an indistinct, rounded quadrilateral manubrium *vs* a thin and smoothly arcuate with a rounded manubrium, gubernaculum wide *vs* thin; from *A. pierci* by spicules that are slightly bent with an indistinct, rounded quadrilateral manubrium *vs* a thin and smoothly arcuate with a rounded manubrium and by a gubernaculum that is wide *vs* thin; from *A. pini* by spicules that are slightly bent and with an indistinct, rounded quadrilateral manubrium *vs* a thin

and smoothly arcuate with a rounded manubrium, and by a gubernaculum that is wide *vs* thin; from *A. robinicola* by spicules with an indistinct, rounded quadrilateral manubrium *vs* a rounded square manubrium and by the length of the male tail spike (ca 33 *vs* >50% of total tail length).

Finally, *A. josephi* n. sp. is distinguished from *A. seani* n. sp. by the morphology of the spicules, which are narrow and tapered (V-shaped) *vs* forked (Y-shaped) in ventral view and have a barely distinct *vs* indistinct manubrium, by the male tail spike, which is less than 25% *vs* fully ca 33% of the total tail length, by the female tail, which is short and conical ($c' = 3.3$ (2.8-4.1) in the stenostomatous form and 3.2 (2.5-3.6) in the eurystomatous form) *vs* elongate conical ($c' = 4.2$ (4.0-4.5) in the stenostomatous form and 3.8 (3.0-5.8) in the eurystomatous form), and by their near full-length SSU sequences, which differ by 13-16 nucleotide positions.

Discussion

REMARKS ON MALE PAPILLA MORPHOLOGY

SEM of the male papillae revealed furcation of v5 and v6 in both new species, consistent with reports for three other *Allodiplogaster* species examined by SEM, namely *A. henrichae*, *A. hirschmannae* and *A. labiomorpha* (Kühne, 1995), all of which belong to the *henrichae* group of *Allodiplogaster* species. Given the long, distinct papillae of species in the *striata* group of *Allodiplogaster* (Kanzaki *et al.*, 2014a), homologous modifications would likely have been reported if present. Therefore, the bifurcation and trifurcation of v5 and v6, respectively, may diagnose the *henrichae* group from other species of *Allodiplogaster*.

Interestingly, similar modifications of v5 and v6 have been found in several different diplogastrid genera (Fig. 19). A split v5 and trifurcate v6, each originating from a socket-like base, have been found in two different clades of *Diplogasteroides* de Man, 1912 (Kiontke *et al.*, 2001; Kanzaki *et al.*, 2013e), *Pseudodiplogasteroides* Körner, 1954 (Kanzaki, unpubl. obs.), and *Mononchoides* Rahm, 1928 (Steel *et al.*, 2011; Atighi *et al.*, 2013). Similar, but not identical, modifications have been reported for several different species in the genera *Parapristionchus* Kanzaki, Ragsdale, Herrmann, Mayer, Tanaka & Sommer, 2012b and *Pristionchus* Kreis, 1932 (Kiontke & Sudhaus, 2000; Kanzaki *et al.*, 2012b, d; Ragsdale *et al.*, 2013a), in which v5 and v6 are both split. In contrast, such

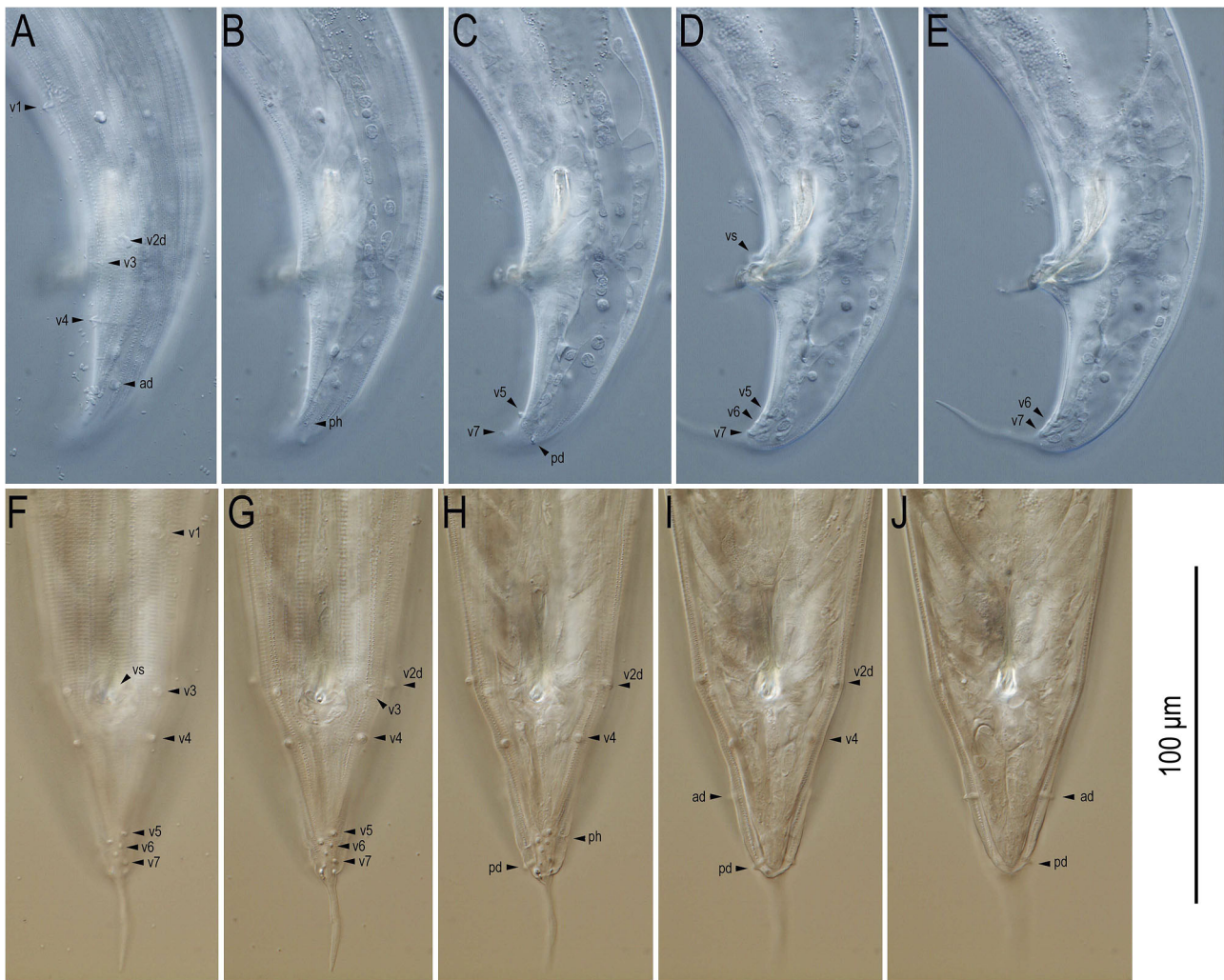


Fig. 16. Male tail region of *Allodiplogaster seani* n. sp. A-E: Left lateral view in different focal planes; F-J: Ventral view in different focal planes. Genital papillae (v + number) and phasmid (ph) are labelled following nomenclature of Sudhaus & Fürst von Lieven (2003). This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/15685411>.

modifications are definitively absent in *Teratodiplogaster* Kanzaki, Giblin-Davis, Davies, Ye, Center & Thomas, 2009a and *Parasitodiplogaster* Poinar, 1979, which are close relatives of *Allodiplogaster* (Giblin-Davis *et al.*, 2006b; Kanzaki *et al.*, 2009a). Among other Diplogastriidae, comparable modifications are also absent in at least *Diplogastrellus* Paramonov, 1952 (Kanzaki *et al.*, 2008), *Rhabditolaimus* Fuchs, 1914 (Susoy & Herrmann, 2012; Kanzaki & Giblin-Davis, 2014), *Rhabditoides* Rahm, 1928 (Kanzaki & Giblin-Davis, 2015), *Oigolaimella* Paramonov, 1952 (Fürst von Lieven, 2003; Fürst von Lieven &

Sudhaus, 2008), *Micoletzkyia* Weingärtner, 1955 (Susoy *et al.*, 2013), *Acrostichus* Rahm, 1928 (Giblin & Kaya, 1984; Kanzaki *et al.*, 2009b), and *Sudhausia* Herrmann, Ragsdale, Kanzaki & Sommer, 2013.

Considering the inferred phylogeny of Diplogastriidae (Susoy *et al.*, 2015), the most parsimonious explanation for the taxonomic distribution of these traits is due to at least four independent gains, namely in: *i*) *Allodiplogaster*; *ii*) a clade including *Mononchoides* but excluding *Sudhausia* (see Susoy *et al.*, 2015); *iii*) a clade including *Diplogasteroides* and *Pseudodiplogasteroides* but

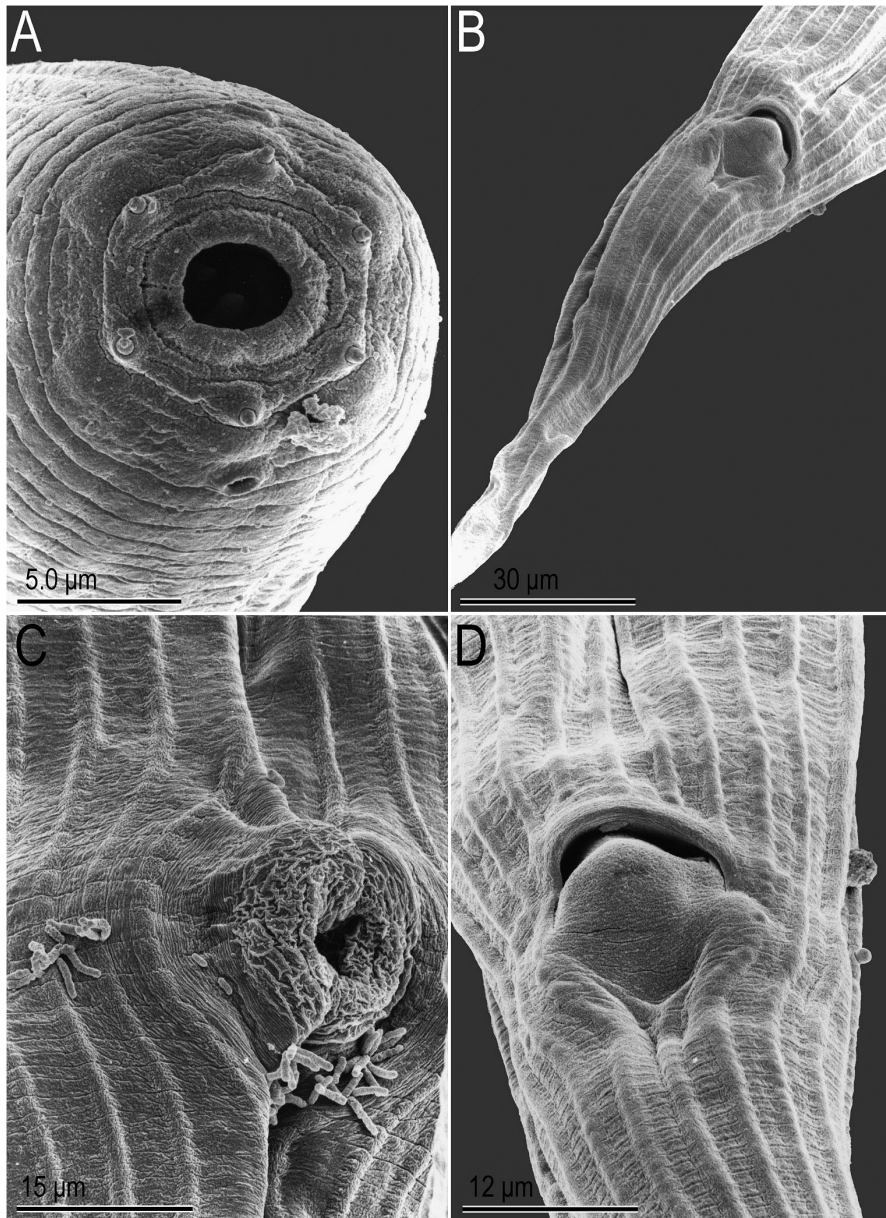


Fig. 17. Scanning electron micrographs of a female stenostomatous form of *Allodiplogaster seani* n. sp. A: En face view; B: Ventral view of tail; C: Ventral view of vulva; D: Ventral view of anus.

excluding *Diplogastrellus* and *Rhabditolaimus*; and iv) a clade including *Pristionchus* and *Parapristionchus* but excluding *Acrostichus*. In the alternative scenario, furcate papillae may have been ancestral to all Diplogastridae excluding *Koerneria* and *Leptojacobus* Kanzaki, Ragsdale, Susoy & Sommer, 2014b but then independently lost

at least seven times. Closer examination of other diplogastrid species, many of which are now available in culture (Susoy *et al.*, 2015), could help elucidate the phylogenetic significance of these traits and we encourage other scientists to provide SEM images when describing or re-describing other diplogastrid species.

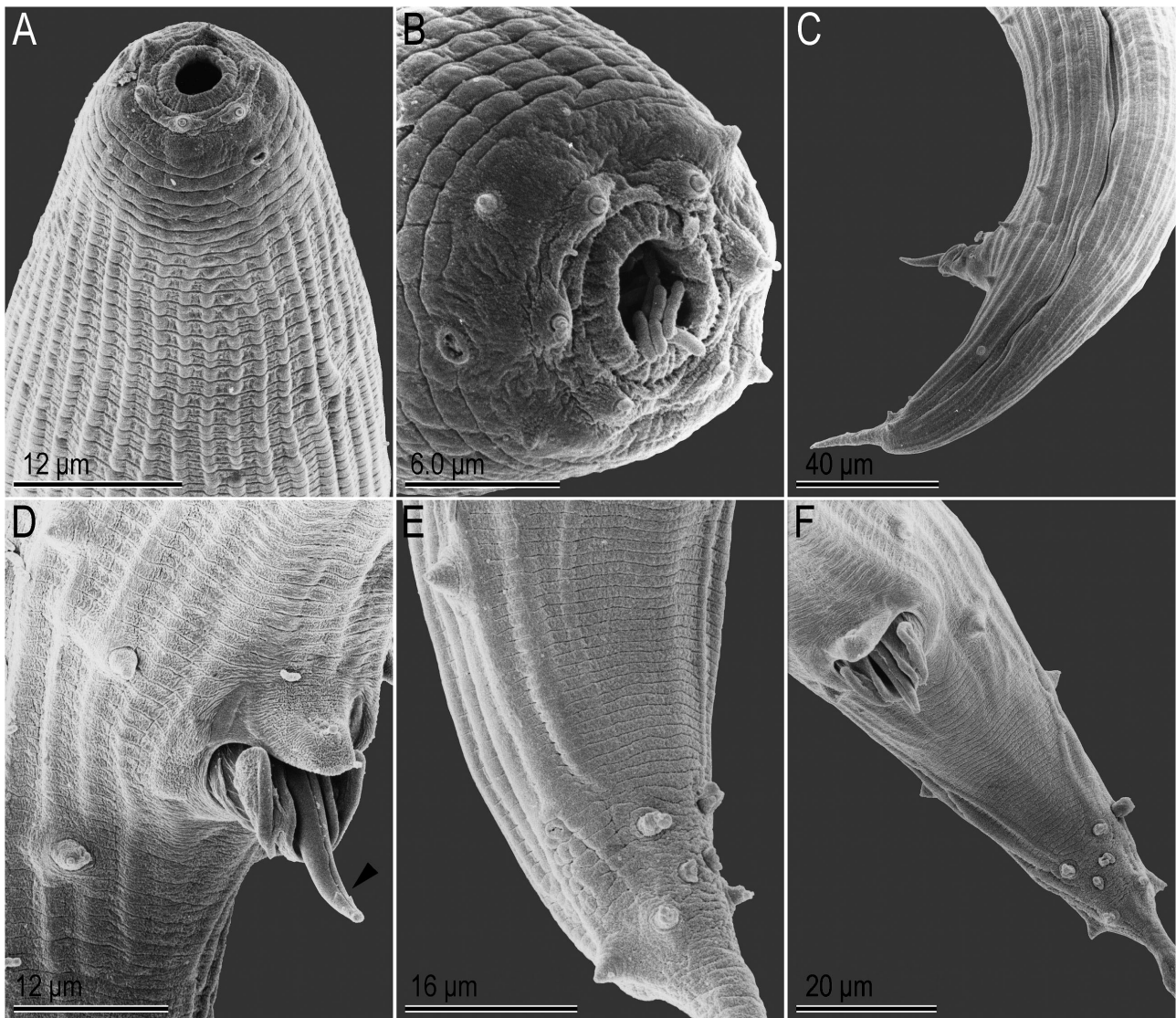


Fig. 18. Scanning electron micrographs of stenostomatous males of *Allodiplogaster seani* n. sp. A: Subventral view of head; B: Near face view; C: Lateral view of posterior body; D: Close-up of cloaca with protracted spicules; E: Subventral close-up of tail tip and papillae; F: Ventral view of tail.

ASSOCIATION OF *ALLODIPLOGASTER* WITH SOIL-DWELLING BEES

The *henrichae* group of *Allodiplogaster* is a repository for terrestrial species in the genus and, except for two soil-inhabiting species, *A. colobocerca* and *A. sudhausi* (Andrássy, 1964; Fürst von Lieven, 2008), species in this group are phoretic on beetles or have been isolated from woody materials infested with insects (summarised in Kanzaki *et al.*, 2014a). For example, *A. henrichae*,

A. hirschmannae and *A. labiomorpha* are associated with *Geotrupes* dung beetles (Coleoptera: Scarabaeidae) (Kühne, 1995); *A. hylobii*, *A. incurva*, *A. pierci* and *A. pini* were isolated from wood-inhabiting *Hylobius* weevils (Curculionidae) (Fuchs, 1915, 1931; Körner, 1954; Massey, 1967); *A. lucani* was isolated from a stag beetle (Lucanidae) (Körner, 1954); *A. histophora* and *A. lepida* were isolated from slime flux, which is associated with many different insects (Weingärtner, 1955; Andrássy,

Table 2. Morphometrics of *Allodiplogaster seani n. sp.* (RGD228). All measurements are in μm and in the form: mean \pm s.d. (range).

Character	Stenostomatous form			Eurystomatous form
	Male		Female	Female
	Holotype	Paratypes	Paratypes	Paratypes
n	–	9	10	10
L	1088	1081 \pm 56 (971-1165)	1430 \pm 42 (1368-1509)	800 \pm 53 (683-859)
a	18.4	18.6 \pm 1.0 (16.3-19.8)	16.1 \pm 1.0 (14.6-17.6)	18.1 \pm 1.3 (16.6-20.7)
b	6.3	6.7 \pm 0.4 (6.2-7.1)	7.9 \pm 0.2 (7.7-8.2)	4.3 \pm 0.3 (4.1-4.9)
c	11.7	12.6 \pm 1.1 (11.4-14.5)	9.8 \pm 0.5 (8.8-10.6)	8.7 \pm 1.6 (5.7-10.1)
c'	2.5	2.5 \pm 0.1 (2.3-2.6)	4.2 \pm 0.2 (4.0-4.5)	3.8 \pm 1.0 (3.0-5.8)
T or V	66.5	65.8 \pm 3.4 (58.4-70.7)	51.0 \pm 1.1 (50.1-54.1)	53.7 \pm 1.9 (50.2-56.0)
Lip diam.	11.1	10.4 \pm 1.0 (8.2-11.7)	11.6 \pm 0.5 (10.5-12.3)	14.0 \pm 1.3 (12.3-15.8)
Stoma width (opening)	3.5	3.2 \pm 0.3 (2.9-3.5)	3.3 \pm 0.3 (2.9-3.5)	5.6 \pm 0.6 (4.1-6.4)
Stoma width (widest part)	5.8	5.1 \pm 0.5 (4.1-5.8)	5.5 \pm 0.4 (4.7-5.8)	9.3 \pm 1.0 (7.6-10.5)
Stoma depth	11.7	11.5 \pm 0.5 (10.5-12.3)	13.0 \pm 0.6 (11.7-14.0)	14.7 \pm 1.3 (12.3-16.4)
Anterior pharynx length	93	90 \pm 2.9 (85-95)	98 \pm 3.6 (94-104)	103 \pm 10.6 (84-117)
Widest part of median bulb from anterior	86	83 \pm 2.4 (80-87)	90 \pm 3.6 (85-96)	95 \pm 9.7 (80-110)
Median bulb diam.	26	26 \pm 1.1 (23-27)	30 \pm 1.4 (28-33)	26 \pm 1.8 (22-29)
Posterior pharynx length	71	66 \pm 4.3 (59-71)	76 \pm 2.5 (73-80)	76 \pm 9.6 (62-85)
Nerve ring from anterior	116	110 \pm 4.3 (106-117)	120 \pm 4.0 (114-125)	123 \pm 12.6 (102-135)
Widest part of basal bulb from anterior	159	149 \pm 5.9 (140-159)	164 \pm 4.4 (159-171)	170 \pm 18.6 (140-191)
Basal bulb diam.	24	24 \pm 1.2 (21-25)	28 \pm 0.8 (26-29)	24 \pm 1.3 (22-27)
Excretory pore from anterior	163	154 \pm 6.4 (144-163)	169 \pm 6.7 (160-182)	142 \pm 15.7 (117-163)
Anterior/posterior pharynx ratio	1.31	1.38 \pm 0.1 (1.24-1.53)	1.29 \pm 0.1 (1.20-1.39)	1.36 \pm 0.1 (1.27-1.56)
Max. body diam.	59	58 \pm 4.7 (51-68)	89 \pm 4.6 (83-97)	45 \pm 4.8 (22-27)
Testis length	724	711 \pm 49 (647-779)	–	–
Anterior ovary length	–	–	499 \pm 35 (441-553)	110 \pm 31 (88-176)
Posterior ovary length	–	–	435 \pm 45 (368-529)	102 \pm 28 (79-169)

Table 2. (Continued.)

Character	Stenostomatous form			Eurystomatous form
	Male		Female	Female
	Holotype	Paratypes	Paratypes	Paratypes
Female rectum length	–	–	43 ± 4.6 (36-51)	30 ± 3.0 (25-33)
Cloacal or anal body diam.	38	35 ± 1.7 (33-38)	35 ± 1.5 (33-38)	25 ± 2.2 (20-28)
Tail length including tail spike	93	86 ± 5.6 (78-93)	149 ± 9.4 (131-166)	95 ± 16.8 (79-136)
Tail spike length	29	29 ± 3.9 (25-39)	–	–
Spicule length (line)	46	44 ± 3.2 (37-49)	–	–
Spicule length (curve)	52	51 ± 3.2 (45-55)	–	–
Gubernaculum length (line)	30	28 ± 2.1 (24-31)	–	–
Female phasmid (distance from anus)	–	–	56 ± 3.9 (48-61)	37 ± 4.9 (30-46)
Female phasmid position (relative position to tail length in %)	–	–	38.1 ± 2.0 (33.9-40.1)	41.6 ± 7.7 (29-49)

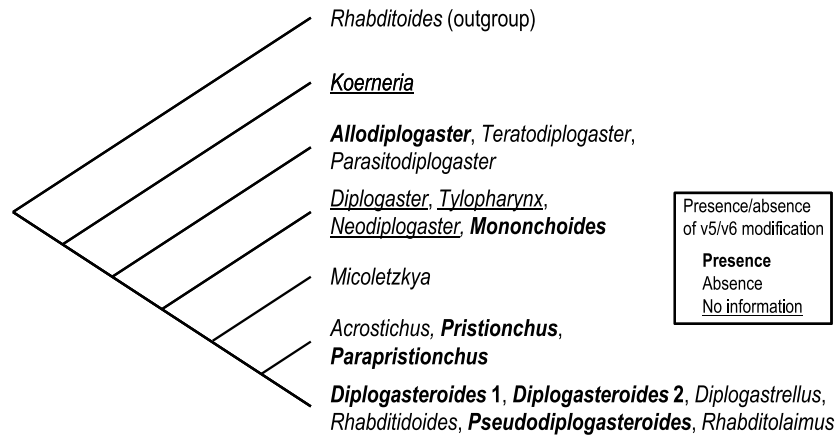


Fig. 19. Taxonomic distribution of split v5 and v6 papillae in Diplogastridae. The tree is summarised from Figure 1.

1958); and *A. robinicola* was isolated from insect frass (Rühm, 1956).

In contrast to the beetle associations of other *henrichae* group species, the insect associations of the two new species appear to be unique among described species, as they were isolated from soil-dwelling bees of the Colletidae and Andrenidae. Interestingly, this association with bees has been found on two different continents, North America (*A. josephi* n. sp. and *A. seani* n. sp.) (Giblin-

Davis *et al.*, 1990) and Europe (*Allodiplogaster* sp. and *A. seani* n. sp.) (Altenkirch, 1962; Hazir *et al.*, 2010). Moreover, an unidentified *Allodiplogaster* was recently collected from *Andrena longitibialis* Hirashima in Hakodate, Hokkaido, Japan (Kanzaki, unpubl. obs.). Thus, this type of association with species of *Allodiplogaster* may be very common in the Holarctic and could have resulted from the univoltine biology of the bees' long-term association with soil. Lending credence to this hypothesis is the col-

lection of *A. seani* n. sp. additionally from the May beetle, *Melolontha melolontha*, grubs of which are also found in the soil. A joint association with scarab beetles and bees is reminiscent of *P. maupasi* which, along with several other unidentified diplogastrids, was isolated from *Andrena optata* Warncke from Turkey (Hazir *et al.*, 2015). Given the widespread association of both *Pristionchus* (Mayer *et al.*, 2009) and *Allodiplogaster* with beetles, the relationship with bees may represent parallel jumps to this new group of hosts from a common soil substrate. Whether or not this secondary association has consequently led to radiations of *Allodiplogaster* with their hosts, as observed between *Acrostichus* (= *Aduncospiculum*) and certain genera of sweat bees (Halictidae) (McFrederick & Taylor, 2013), needs to be tested by broader sampling of potential bee hosts.

A similar pattern of insect-associated diversity has been reported in *Bursaphelenchus* Fuchs, 1937, which are fungal-feeding nematodes and often entomophilic. Most species of that genus are associated with various groups of beetles, namely bark beetles and ambrosia beetles (Curculionidae: Scolytinae, Platypodinae), weevils, longhorn beetles (Cerambycidae), sap beetles (Nitidulidae) and stag beetles (Ryss *et al.*, 2005; Giblin-Davis *et al.*, 2006a; Kanzaki *et al.*, 2011a, b, 2012d). However, *Bursaphelenchus* contains several species associated with hymenopteran insects, including soil-dwelling bees and fig wasps, namely: *B. abruptus* Giblin-Davis, Mundo-Ocampo, Baldwin, Norden & Batra, 1993 associated with *Anthophora abrupta* Say (Giblin-Davis *et al.*, 1993); *B. anatolius* Giblin-Davis, Hazir, Center, Ye, Keskin, Thorp & Thomas, 2005 associated with *Halictus* spp. (Giblin-Davis *et al.*, 2005); *B. debrae* Hazir, Giblin-Davis, Keskin, Ye, Kanzaki, Center, Hazir, Kaya & Thomas, 2007 associated with *H. brunnescens* (Eversmann) (Hazir *et al.*, 2007); *B. kevinci* Giblin, Swan & Kaya, 1984 associated with *Halictus* spp. (Giblin *et al.*, 1984); *B. seani* Giblin & Kaya, 1983 associated with *Anthophora bomboidea stanfordiana* Cockerell (Giblin & Kaya, 1983); and *B. sycophilus* Kanzaki, Tanaka, Giblin-Davis & Davies, 2014e associated with *Ceratosolen appendiculatus* (Mayr) (Kanzaki *et al.*, 2014e). As in Diplogastridae, hymenopteran associations of *Bursaphelenchus* have evolved several times independently (Kanzaki *et al.*, 2014c), followed by apparent radiations in the *B. kevinci* group, associated with halictids (*B. anatolius*, *B. debrae* and *B. kevinci*), and in the *B. sycophilus* group, associated with fig wasps that pollinate the *Sycomorus* group of *Ficus*. In addition to *Bursaphelenchus*, several other

nematode genera have been reported in association with soil-dwelling bees from different parts of the world, e.g., *Ditylenchus halictus* Giblin-Davis, Erteld, Kanzaki, Ye, Zeng & Center, 2010, a species from a genus of plant-parasitic and mycophagous nematodes that was isolated from *Halictus sexcinctus* (Fabricius) from Germany, and a mycophagous aphelenchid, *Aphelenchoides xylocopae* Kanzaki, 2006, that was isolated from the carpenter bee, *Xylocopa appendiculata circumvolans* Smith from Japan (Kanzaki, 2006). Although the origins of these associations are in most cases not clear, associations with soil, or possibly other moist habitats, followed by host carrier switching appear to be an important means for the evolution of these interesting entomophilic nematodes.

Acknowledgements

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