

# *Sudhausia aristotokia* n. gen., n. sp. and *S. crassa* n. gen., n. sp. (Nematoda: Diplogastridae): viviparous new species with precocious gonad development

Matthias HERRMANN<sup>1</sup>, Erik J. RAGSDALE<sup>1</sup>, Natsumi KANZAKI<sup>2</sup> and Ralf J. SOMMER<sup>1,\*</sup>

<sup>1</sup>Max Planck Institute for Developmental Biology, Department of Evolutionary Biology,  
Spemannstraße 37, Tübingen, Germany

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

Received: 27 March 2013; revised: 9 May 2013

Accepted for publication: 9 May 2013; available online: 3 July 2013

**Summary** – Two new species of diplogastrid nematodes, *Sudhausia aristotokia* n. gen., n. sp. and *S. crassa* n. gen., n. sp., were isolated from dung beetles (Coleoptera: Scarabaeidae) in Ghana and South Africa, respectively. *Sudhausia* n. gen. is circumscribed by stomatal morphology, namely the presence of a tube-shaped gymnostom, a pair of conical subventral denticles in the metastegostom, and a disk-like telostegostom bearing minute conical denticles. Other characters distinguishing the new genus are the presence of a bursa in the male and a vulva lined anteriorly and posteriorly by columns of sac-like cells. The two new species, both of which are hermaphroditic, are distinguished from each other by spicule and gubernaculum morphology, male papillae arrangement, bursal shape, vaginal morphology, and phasmid position. A suite of unusual developmental traits that distinguishes *Sudhausia* n. spp. includes maturation of the gonad and development of juvenile progeny before moulting to adulthood, a two-fold increase in embryo size during development, and constitutive vivipary. A phylogeny inferred from 11 ribosomal protein-coding genes and a fragment of the small subunit rRNA gene show *Sudhausia* n. gen. to be divergent from other sequenced diplogastrid taxa, including those characterised by a tube-like stoma. The two new species represent useful new reference points for the study of feeding-structure evolution in Diplogastridae.

**Keywords** – androdioecy, description, dung beetles, Ghana, hermaphrodite, molecular, morphology, morphometrics, new genus, new species, *Onthophagus*, phylogeny, Scarabaeidae, *Scarabaeus*, South Africa, stoma, taxonomy.

The nematode family Diplogastridae Micoletzky, 1922 currently includes 31 genera (Sudhaus & Fürst von Lieven, 2003; Kanzaki *et al.*, 2009, 2012a; Mayer *et al.*, 2009; Fürst von Lieven *et al.*, 2011; Susoy & Herrmann, 2012), many of which are associated with insects. Consequently, intensive sampling of potential insect hosts has recently revealed diverse new taxa in the family (Kanzaki *et al.*, 2009, 2012a). Scarab beetles (Coleoptera: Scarabaeidae) in particular are hosts to many diplogastrid species, especially in *Pristionchus* Kreis, 1932 (Herrmann *et al.*, 2006; Mayer *et al.*, 2007, 2009; Rae *et al.*, 2008; Kanzaki *et al.*, 2012a, 2013), but also in *Diplogasteroides* de Man, 1912, *Koerneria* Meyl, 1960, *Mononchoides* Rahm, 1928, *Rhabditoides* Rahm, 1928, and *Tylopharynx* de Man, 1876 (Mayer *et al.*, 2009).

Given the diversity of feeding structures in diplogastrids (Fürst von Lieven & Sudhaus, 2000; Sudhaus & Fürst von Lieven, 2003), the isolation of new higher order taxa in the family is particularly valuable for studies of the evolution of form. In addition to the presence of detailed references for diplogastrid stomatal anatomy (Baldwin *et al.*, 1997; Bumbarger *et al.*, unpubl.), the established model system *Pristionchus pacificus* Sommer, Carta, Kim & Sternberg, 1996 allows genetic and developmental mechanistic studies of morphology (Bento *et al.*, 2010; Sommer & McLaughran, 2013). The potential to extend studies in *Pristionchus* to other species of diplogastrids, especially those amenable to laboratory culture, is made more powerful by the discovery of new forms to include in a comparative context. Here, we describe a

\* Corresponding author, e-mail: ralf.sommer@tuebingen.mpg.de

new genus of Diplogastridae, *Sudhausia* n. gen., based on its unique stomatal morphology, sex-specific characters, and a suite of unusual developmental traits. Reflecting the frequent association of diplogastrids with scarab beetles, both of the new species, *S. aristotokia* n. gen., n. sp. and *S. crassa* n. gen., n. sp., were recorded from dung beetles in Ghana and South Africa, respectively.

## Materials and methods

### NEMATODE ISOLATION AND CULTIVATION

*Sudhausia aristotokia* n. gen., n. sp. was isolated from adult dung beetles as detailed for individual species below. Host beetles were dissected on a 2.0% agar plate, after which the plate was kept at room temperature for several weeks. Nematodes proliferated on bacteria associated with the host beetle cadavers. Individuals were thereafter transferred to nematode growth medium (NGM) agar plates seeded with the original microbes from the beetle carcass, and they have been since kept in laboratory culture on this medium.

### MORPHOLOGICAL OBSERVATION AND PREPARATION OF TYPE MATERIAL

One- to two-week-old cultures of *S. aristotokia* n. gen., n. sp. and *S. crassa* n. gen., n. sp. provided material for morphological observation. Observations by light microscopy (LM) and differential interference contrast (DIC) microscopy were conducted using live nematodes, which were hand-picked from culture plates. Because of the clarity and integrity of its morphology, live material was used for morphometrics. For line drawings, specimens were mounted into water on slides and then relaxed by applying gentle heat. For morphometrics, specimens were mounted on slides with pads of 5% noble agar and 0.15% sodium azide and were additionally relaxed by heat when necessary. To prepare type material, nematodes were isolated from type strain cultures, rinsed in distilled water to remove bacteria, heat-killed at 65°C, fixed in TAF to a final concentration of 5% formalin and 1.5% triethanolamine, and processed through a glycerin and ethanol series using Seinhorst's method (see Hooper, 1986). Alternatively, some paratypes were fixed in 5% formalin after heat killing at 65°C. Nomarski micrographs were taken using a Zeiss Axio Imager Z.1 microscope and a Spot RT-SE camera supported by the program MetaMorph v.7.1.3 (Molecular Devices).

### SCANNING ELECTRON MICROSCOPY

Nematodes were prepared for scanning electron microscopy (SEM) by fixation in 2.5% glutaraldehyde in M9 buffer and then post-fixed with 1% osmium tetroxide. After several rinses with water, samples were dehydrated through a graded ethanol series, followed by critical point drying using carbon dioxide. Specimens were mounted on polylysine-coated cover slips, sputter-coated with 20 nm gold/palladium, and then imaged with a Hitachi S-800 field emission scanning electron microscope operating at 20 kV.

### MOLECULAR CHARACTERISATION AND PHYLOGENY

For species diagnosis and phylogenetic analysis, we amplified and sequenced an approximately 500-bp fragment of the small subunit (SSU) rRNA gene of the two new species using the primers SSU18A (5'-AAAGATTAAGCCATGCATG-3') and SSU9R (5'-AGCTGGAATTA CCGCGGCTG-3') (Floyd *et al.*, 2002). Partial SSU rRNA sequences of *S. aristotokia* n. gen., n. sp. and *S. crassa* n. gen., n. sp. have been deposited in the GenBank database under accession numbers KC831555 and KC831556, respectively. Additionally, we amplified and sequenced 11 ribosomal protein-coding genes for the two new species: *rpl-2*, *rpl-6*, *rpl-9*, *rpl-10*, *rpl-14*, *rpl-23*, *rpl-35*, *rps-7*, *rps-14*, *rps-27* and *rps-28*. All information regarding genes, primers and PCR conditions is given in Mayer *et al.* (2009). Except for *rps-28*, the sequence of which was less than 200 bp, all ribosomal protein gene sequences for *S. aristotokia* n. gen., n. sp. and *S. crassa* n. gen., n. sp. have been deposited in GenBank under accession numbers KC831557-KC831566 and KC831567-KC831576, respectively. Phylogenetic analysis included species from all diplogastrid genera for which homologous sequences have been published (Mayer *et al.*, 2009; Kanzaki *et al.*, 2012a).

The concatenated dataset of ribosomal protein genes and the partial SSU rRNA gene was aligned using MUSCLE (Edgar, 2004), followed by manual alignment in MEGA5.10 (Tamura *et al.*, 2011), which included deletion of ambiguous alignment positions. The final alignment comprised 5963 positions. The alignment was partitioned into four subsets: one for the partial SSU rRNA gene and three according to codon position for the concatenated set of ribosomal protein genes. Analyses by maximum likelihood (ML) and Bayesian inference, as implemented in RAxML v.7.2.8 (Stamatakis, 2006) and MrBayes 3.2 (Ronquist *et al.*, 2012), respectively,

were used to infer the phylogeny. Fifty independent runs were performed for the ML analysis, in which trees were inferred under a general time reversible model with a gamma-shaped distribution of rates across sites. Bootstrap support was calculated by 1000 pseudoreplicates on the most likely tree among all runs. Bayesian analyses were initiated with random starting trees and were run with four chains for  $4 \times 10^6$  generations. Markov chains were sampled at intervals of 100 generations. Two independent runs were performed for the analysis. After confirming convergence of runs and discarding the first  $2 \times 10^6$  generations as burn-in, remaining topologies were used to generate a 50% majority-rule consensus tree with clade credibility values given as posterior probabilities (PP). Bayesian analysis invoked a mixed model of substitution with a gamma-shaped distribution across sites and, as informed by previously studies (Mayer *et al.*, 2009; van Megen *et al.*, 2009), specified *Rhabditoides inermis* (Schneider, 1866) Dougherty, 1955 as outgroup. Model parameters were unlinked across character partitions in both ML and Bayesian analyses.

## Results

### *Sudhausia*\* n. gen.

#### DESCRIPTION

Diplogastridae. Body cylindrical, stout. Cuticle with fine annulation, fine striations observed by LM but not appearing on surface, and one fine lateral line appearing on surface on each side, only visible by SEM. Lips fused in pairs (one dorsal, two subventral) and separated by thin rims of labial cuticle, resulting in an ypsiliform stomatal opening. Six short, papilliform labial sensilla present in both males and morphological females (only hermaphrodites are known) and four papilliform cephalic papillae present in males, as typical for diplogastrid nematodes. Labial and cephalic papillae visible by LM. Cheilostom triradiate, anterior rim of which punctuated by grooves corresponding to separation of lips. Gymnostom tube-shaped, twice as long as wide, divided into two distinct regions: *i*) an offset, short anterior region of smooth cuticle with a barely distinguishable midline separating an anterior rim that is slightly thicker than the

posterior rim; and *ii*) a longer posterior region with cuticle refracting to give appearance of a rough texture. Stegostom comprising: *i*) a transversely flat rim of cuticle (pro-/mesostegostom) at anterior margin of muscular pharynx; *ii*) a cylindrical metastegostom bearing a moveable dorsal tooth, dorsoventrally narrowed for its entire length and transversely triangular, with a clear gland duct and orifice and, in each of subventral sectors, a pair of narrow, conical, equally sized and axially oriented denticles, all borne from a level slightly posterior to dorsal tooth; and *iii*) a disk-like telostegostom with an anterior rim and bearing minute, conical, axially oriented denticles arranged in three pairs (one dorsal, two subventral). Apodemes absent. Procorpus and metacarpus muscular. Metacarpus similar to, or slightly wider than, procorpus in diam. Isthmus narrow, not muscular. Basal bulb glandular. Pharyngo-intestinal junction clearly observed, well developed. Morphological females amphidelphic, ovotestes reflexed. Vagina surrounded anteriorly and posteriorly by columns of sac- or pouch-like cells, largest being those closest to ventral body surface. Bursa present. Male with 8-9 pairs of genital papillae, those on bursa forming rays, and one small, unpaired, ventral genital papilla on anterior cloacal lip.

#### BIONOMICS

In addition to the above morphological characters, which definitively circumscribe *Sudhausia* n. gen., the following biological traits distinguish and are considered tentatively diagnostic of the genus. Species are androdioecious, consisting of males and self-fertile hermaphrodites. Somatic gonad of hermaphrodites develops precociously. Developing embryos occur inside the gonad during the early J4 stage of the mother, *i.e.*, when the vulva is beginning to invaginate. Gonads in older hermaphrodites appear disorganised due to the presence of growing embryos and hatching juveniles. Embryos lack a discernible eggshell, are irregular in shape, and grow two-fold in size from the zygote to the comma stage. Dauer juveniles are often present in the intra-uterine brood. Primary branches of gonads in mature adults are therefore filled with a brood of mixed developmental stages that together occupy most of the body volume of the living, active mother. Internal non-dauer juveniles have been seen to feed on vesicles of unknown origin and substance. Juveniles are born constitutively by vivipary, even under well-fed laboratory conditions.

\* Named in honour of Prof. Walter Sudhaus (Freie Universität Berlin, Berlin, Germany), in recognition of a career of outstandingly detailed work in rhabditid and diplogastrid morphology and systematics.

#### TYPE SPECIES

*Sudhausia aristotokia* n. gen., n. sp.

#### OTHER SPECIES

*Sudhausia crassa* n. gen., n. sp.

#### RELATIONSHIPS

*Sudhausia* n. gen. is distinguished from all other Diplogastriidae by the combined presence of a tube-shaped (*i.e.*, 'rhabditiform') gymnostom and a pair of conical denticles in the subventral metastegostom and in all sectors of the telostegostom (Fürst von Lieven & Sudhaus, 2000; Sudhaus & Fürst von Lieven, 2003). The new genus is therefore distinguished from all other diplogastrid genera with a tube-like gymnostom, namely *Demaniella* Steiner, 1914, *Diplogasteroides*, *Diplogastrellus* Paramonov, 1952, *Goffartia* Hirschmann, 1952, *Mehdinema* Farooqui, 1967, *Pseudodiplogasteroides* Körner, 1954, *Rhabditoides*, and *Rhabditolaimus* Fuchs, 1914, by the presence of this armature. Also unique to *Sudhausia* n. gen. is a vagina lined anteriorly and posteriorly by columns of sac-like cells. The similar diameter of the procorpus and metacarpus distinguishes *Sudhausia* n. gen. from other diplogastrids except for *Rhabditolaimus* and *Odontopharynx* de Man, 1912, the latter being distinguished from *Sudhausia* n. gen. by its barrel-shaped and heavily cuticularised gymnostom and large, dagger-like dorsal tooth. The presence of a bursa distinguishes *Sudhausia* n. gen. from other diplogastrids except for *Heteropleuronema* Andrassy, 1970 and some species of *Neodiplogaster* Cobb, 1924 and *Rhabditolaimus*, all of which are clearly distinguished from *Sudhausia* n. gen. by stomatal and pharyngeal morphology. Finally, heterochronic gonad development and growing embryos both distinguish *Sudhausia* n. gen. from all other diplogastrid genera.

#### MOLECULAR CHARACTERISATION AND PHYLOGENY

The concatenated alignment comprised 2342 parsimony-informative sites. Phylogenetic analysis of sequences of the SSU rRNA and 11 ribosomal protein loci revealed divergence of *S. aristotokia* n. gen., n. sp. and *S. crassa* n. gen., n. sp. from each other and from all other diplogastrid genera for which both SSU rRNA and orthologous ribosomal protein gene sequences were available (Figs 1, 2).

The diagnostic 450 bp fragment of SSU rRNA in particular differed by ten nucleotide positions, supporting their identity as two unique species.

Topologies inferred by ML (Fig. 1) and Bayesian (Fig. 2) analyses differed in several deep, poorly supported nodes, but the trees were congruent in several respects. Firstly, *S. aristotokia* n. gen., n. sp. and *S. crassa* n. gen., n. sp. were fully supported as sister taxa (100% BS, PP). Secondly, *Sudhausia* n. gen. was shown to be a sister group to a clade comprising *Tylopharynx* sp. + *Neodiplogaster* sp. + *Mononchoides* sp., albeit with variable support (62% BS, 100% PP). Thirdly, a separate clade including *Diplogastrellus gracilis* Bütschli, 1876 + *Rhabditoides* sp. and *Pseudodiplogasteroides* sp. + *Diplogasteroides* spp. was moderately or well supported as monophyletic (83% BS, 100% PP). Finally, *Sudhausia* n. gen. was nested within a well supported clade (100% BS, PP) that included all analysed diplogastrids other than *Koerneria* spp. Differing between the two analyses was the position of *Rhabditolaimus* sp. + *Oigolaimella attenuata* Fürst von Lieven & Sudhaus, 2008, which in both cases was inferred to have a negligibly supported relationship to their sister group (<50% BS, 65% PP). The relationship of a clade comprising *Sudhausia* n. gen., *Tylopharynx* sp., *Neodiplogaster* sp., and *Mononchoides* sp. to other lineages was also unresolved, being poorly supported and differing between analyses.

#### ***Sudhausia aristotokia*\* n. gen., n. sp.** (Figs 3-6; 7A-C, E; 8A-C)

#### MEASUREMENTS

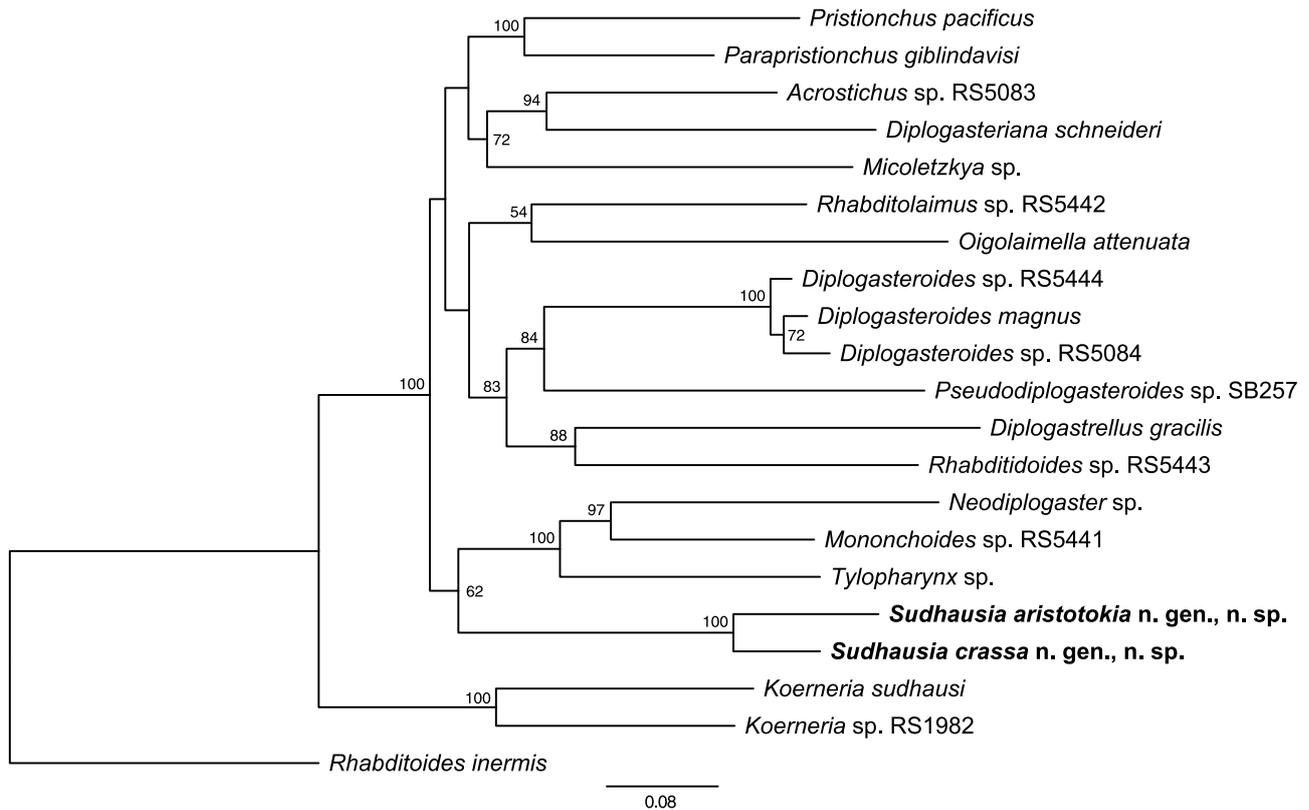
See Table 1.

#### DESCRIPTION

##### *Adults*

Labial region slightly offset, lips squared or acutely angular at junction of anterior and outer lateral walls. Amphidial apertures elliptical, located subdorsally at level of posterior cheilostom. Dorsal pharyngeal gland clearly observed, penetrating dorsal tooth to gland opening. Procorpus occupying half to two-thirds of corresponding body diam. Metacarpus similar to, or slightly wider than,

\* Derived from the Greek ἄριστος ('best') + τόκος ('birth') and refers to the vivipary of this species.



**Fig. 1.** Phylogenetic relationships of Diplogastridae, including *Sudhausia aristotokia* n. gen., n. sp. and *S. crassa* n. gen., n. sp., inferred by maximum likelihood (ML) from an approximately 450 bp fragment of the SSU rRNA gene and 11 ribosomal protein-coding genes. The tree with the highest log-likelihood ( $-55811.647527$ ) is shown. The percentage of trees in which the associated taxa clustered together in 1000 bootstrap pseudoreplicates is shown next to the nodes (left value). Bootstrap support values above 50% are shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

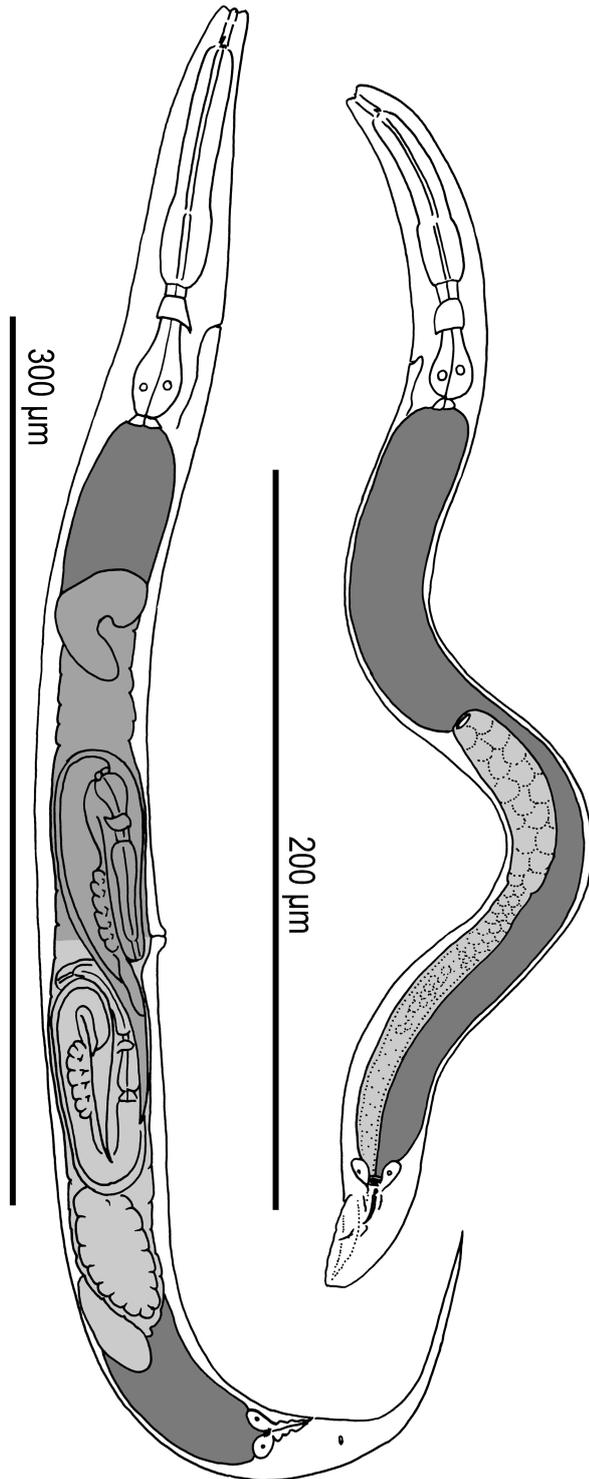
procorpus in diam. Corpus twice as long as postcorpus. Nerve ring surrounding anterior part to mid-region of isthmus. Excretory pore located at level of mid-isthmus to anterior part of basal bulb. Hemizonid not clearly observed. Deirid at level of, or posterior to, pharyngo-intestinal valve.

#### Male

Spontaneous (= fatherless) males rare in culture (<1%). Testis single, located along ventral side. Spermatogonia arranged in *ca* three rows in reflexed part, in two to three rows in anterior half of main branch, then mature amoeboid spermatids arranged in multiple rows in remaining, proximal part of gonad. *Vas deferens* not clearly separated from other parts of gonad. Spicules paired, separate. Spicules long, slender, appearing pliable (bending inconsistently) in ventral view, adjacent to each other for

distal fifth of their length. Spicule in lateral view smoothly and ventrally arcuate, giving spicule curvature of *ca* 135°. Manubrium squared, 1.5-2.0 times as long as wide, twice as wide as spicule blade (= lamina/calomus complex) in ventral view. Spicule blade only slightly expanded at one fourth of spicule length from anterior end. Gubernaculum half of spicule length long, slender, with medially split, knob-like anterior tip, anterior half not recurved with respect to posterior half, posterior half offset from anterior half and forming a tube enveloping spicules. Cloacal opening dome-shaped in ventral view. Three cloacal glands, two subventral and one dorsal, present but not obvious. Two pairs of genital papillae ventral ('vn') to alae, six pairs forming bursal rays, ending distinctly on dorsal side ('Rnd'), ventral side ('Rnv'), or margin ('Rn') of bursa (nomenclature is original, as homologies with papillae of other diplogastrids are ambiguous). Pairs of papillae





**Fig. 3.** Mature adults of *Sudhausia aristotokia* n. gen., n. sp. Left, hermaphrodite, right lateral view. Right, male, left lateral view.

sacs appear membranous. Vulva slightly to strongly protruberant in lateral view, pore-like in ventral view. Rectum *ca* 1 anal body diam. long, intestinal-rectal junction surrounded by well developed sphincter muscle. Three rectal glands, two subventral and one dorsal, present. Anus in form of dome-shaped slit, posterior anal lip slightly protruberant. Phasmid conspicuous, located *ca* 0.5 anal body diam. posterior to anus. Tail conical, *ca* 4-5 anal body diam. in length, with conical terminus.

#### TYPE HOST (CARRIER) AND LOCALITY

The type strain was established from an individual nematode isolated from the body of a dung beetle of the genus *Onthophagus* Latreille, 1802 (Coleoptera: Scarabaeidae) in Borbiri, Ghana. Only one of eight beetles examined was positive for the nematode.

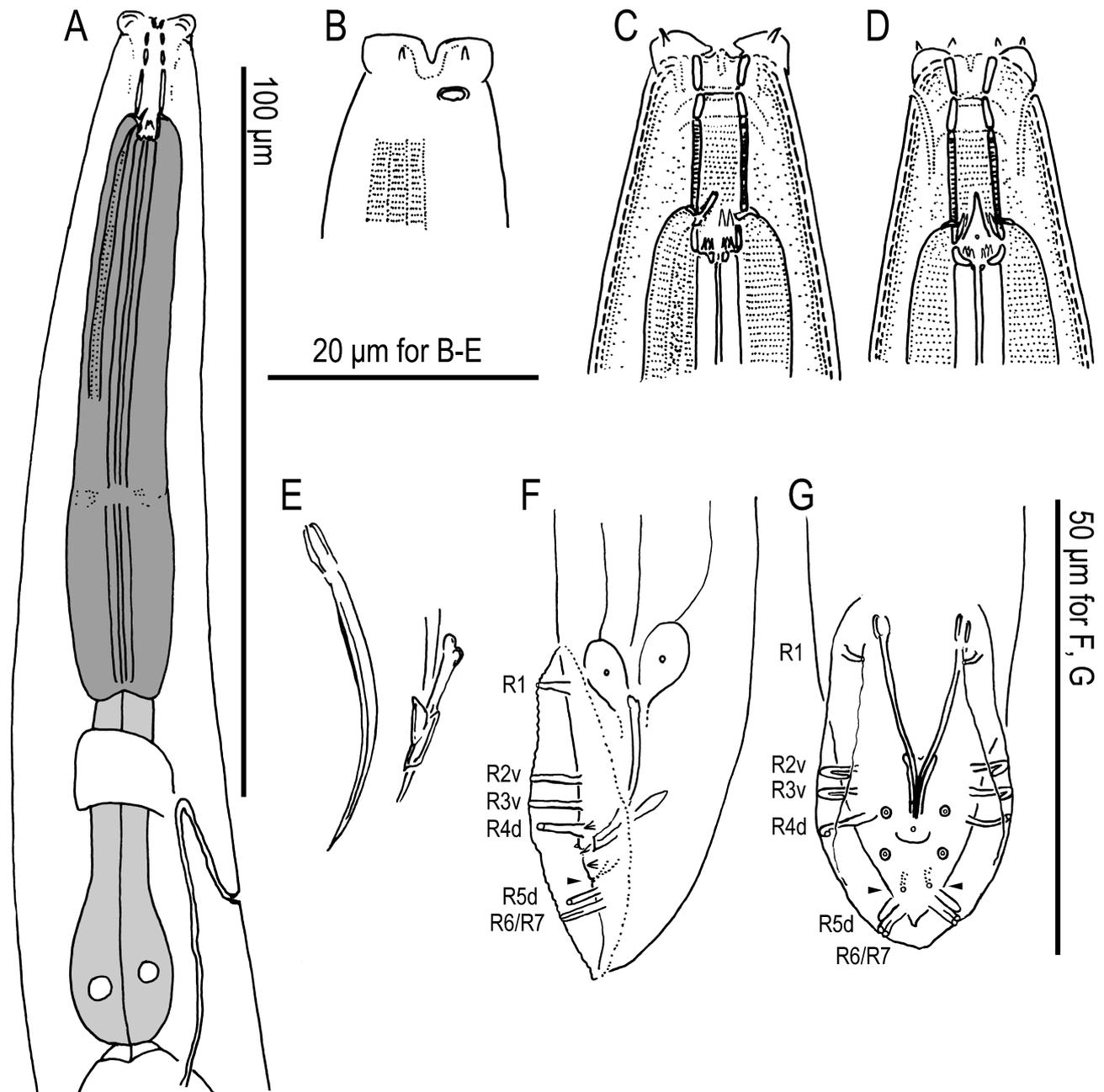
#### TYPE MATERIAL AND STRAIN CULTURE

Holotype male, two paratype males, and six paratype hermaphrodites deposited in the University of California Riverside Nematode Collection (UCRNC), Riverside, CA, USA. Four paratype hermaphrodites deposited in the Natural History Museum Karlsruhe, Karlsruhe, Germany. The type strain is available in living culture from the Department of Evolutionary Biology, Max Planck Institute (MPI) for Developmental Biology, Tübingen, Germany, and can be provided to other researchers upon request. Attempts to achieve frozen stocks of this species have been unsuccessful.

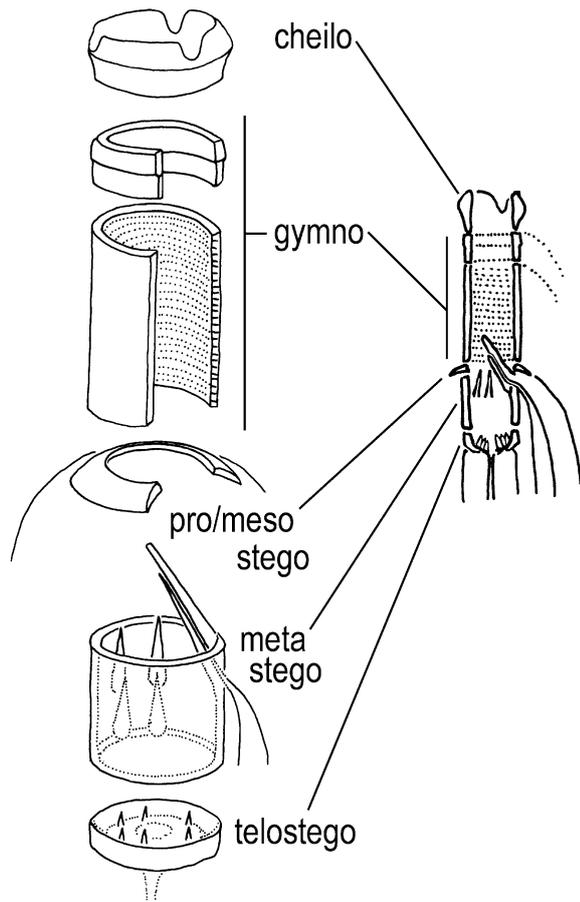
#### DIAGNOSIS AND RELATIONSHIPS

*Sudhausia aristotokia* n. gen., n. sp. is diagnosed by the long and thin spicules with no ventral expansion of the spicule blade (*i.e.*, calomus is not offset from lamina), a straight and narrow gubernaculum, the male genital papillae arranged as (R1, R2v, R3v, (R4d, v1), C, v2, (R5d, Ph, R6, R7)), whereby R6 and R7 emerge from a common opening, and an offset labial region with squared or acutely angular lips.

The species is distinguished from its only known congener, *S. crassa* n. gen., n. sp., by the following characters: spicules that are long, thin with a manubrium twice as wide as blade transversely *vs* spicules that are robust with a manubrium only 1.5 times wide as blade transversely; a straight and narrow *vs* stout and recurved gubernaculum; a bursa with alae extending 0.5 cloacal body diam. *vs* less than 0.33 cloacal body diam. from the



**Fig. 4.** *Sudhausia aristotokia* n. gen., n. sp. A: Neck region of hermaphrodite, right lateral view; B: Lip region of male, left lateral view; C: Stomatal region of hermaphrodite, right lateral view; D: Stomatal region of hermaphrodite, ventral view; E: Spicule and gubernaculum, left lateral view; F: Male tail, left lateral view; G: Male tail, ventral view. Abbreviations: R1-R7 = bursal ray papillae; d = dorsal; v = ventral.



**Fig. 5.** Schematic representation of the stoma of *Sudhausia aristotokia* n. gen., n. sp. Abbreviations: cheilo = cheilostom; gymno = gymnostom; pro/meso stego = pro-/mesostegostom; meta stego = metastegostom; telostego = telostegostom.

body; male genital papillae arranged as (R1, R2v, R3v, (R4d, v1), C, v2, (R5d, Ph, R6, R7)) vs (R1, R2, R3d, (v1, C), v2, (Ph, R4d) (R5d, R6v)); by R1 far (almost one cloacal body diam.) anterior to R2v, which is close to R3v vs R1 closer to R2 than is R3d; C equidistant from v1 and v2 vs at same level as v1; single papillae of v1 as close to each other as are single papillae of v2 vs further from each other than single papillae of v2; R7 fused to or emerging from a common opening with R6 vs R7 absent; and a corpus that is twice as long vs 1.5 times as long as the postcorpus. Hermaphrodites of *S. aristotokia* n. gen., n. sp. are distinguished from those of *S. crassa* n. gen., n. sp. by a phasmid that is anterior (by 0.33 anal body diam.) vs posterior (by 0.5 anal body diam.) to the anus and by a vagina lined by sac-like cells that have a membranous vs thickened appearance. Both sexes of

*S. aristotokia* n. gen., n. sp. can be distinguished from those of *S. crassa* n. gen., n. sp. by an offset labial region with squared or acutely angular lips vs a labial region that is rounded and not offset. Although body shape can vary substantially with age and among individuals within species, *S. aristotokia* n. gen. n. sp. has a stout, vs fat, very stout, body and by a longer (4-5 anal body diam.) and narrowly conical vs shorter (3-4 anal body diam.), widely conical tail. Finally, each species is diagnosed by a unique sequence in a 450 bp fragment of SSU rRNA, which differs by ten positions between the two species.

***Sudhausia crassa*\* n. gen., n. sp.**  
(Figs 7D-H; 8D, F-H; 9, 10)

MEASUREMENTS

See Table 1.

DESCRIPTION

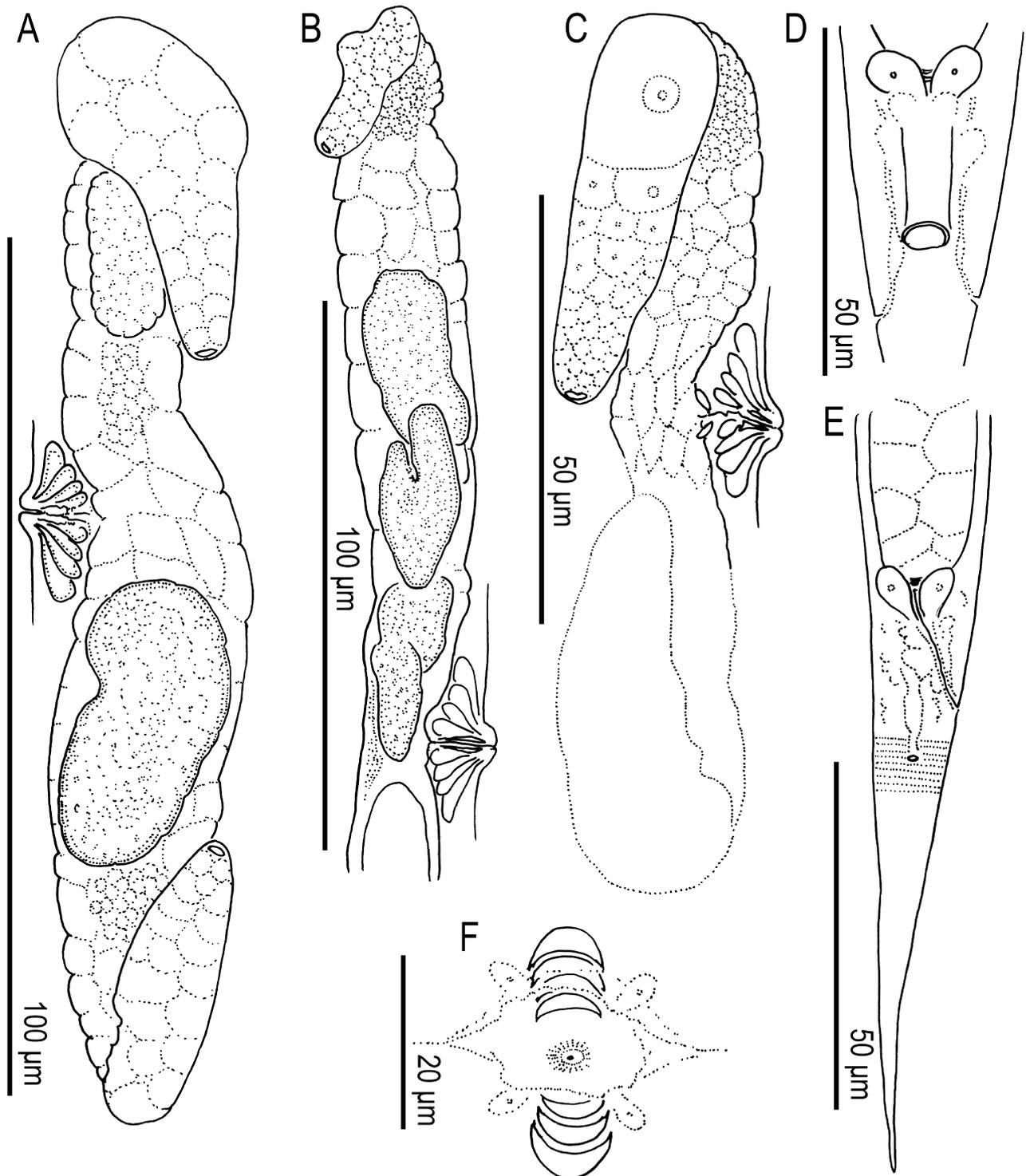
*Adults*

Labial region not offset, lips rounded at junction of anterior and outer lateral walls. Amphidial apertures elliptical, located subdorsally at level of posterior cheilostom. Dorsal pharyngeal gland clearly observed, penetrating dorsal tooth to gland opening. Procorpus occupying half to two-thirds of corresponding body diam. Metacorpus similar in diam. to, or slightly wider than, procorpus. Corpus 1.5 times as long as postcorpus. Nerve ring surrounding anterior to mid-isthmus. Excretory pore located at level of mid-isthmus to anterior part of basal bulb. Hermizoid not clearly observed. Deirid posterior to pharyngo-intestinal valve.

*Male*

Spontaneous males rare in culture (<1%). Body stout. Testis single, located along ventral side, anterior part reflexed to either right or left side. Spermatogonia arranged in ca three rows in reflexed part, in 2-3 rows in anterior half of main branch, then mature amoeboid spermatids arranged in multiple rows in remaining, proximal part of gonad. Vas deferens not clearly separated from other parts of gonad. Spicules paired, separate. Spicules robust in ventral view, adjacent to each other for distal fifth of their

\* Latin adjective meaning 'fat, stout' and referring to the general body shape of mature adults, especially hermaphrodites.



**Fig. 6.** Young hermaphrodites of *Sudhausia aristotokia* n. gen., n. sp. A-C: Variation in reproductive tract among individuals. A: Left lateral view; B: Right lateral view; C: Right lateral view; D: Anus, ventral view; E: Tail region, right lateral view; F: Vulva, ventral view.

length. Spicule in lateral view smoothly and ventrally arcuate, giving a spicule curvature of *ca* 135°. Manubrium ovoid, less than 1.5 times as long as wide, only slightly wider than spicule blade in ventral view. Spicule blade distinctly expanded at one-third of spicule length from anterior end. Gubernaculum 0.5 spicule lengths long, robust, broad anteriorly such that dorsal wall slightly recurved and dorsal and ventral walls separate at a 60° angle at posterior end, posterior half not offset from anterior half, forming a tube enveloping spicules. Cloacal opening dome-shaped in ventral view. Three cloacal glands, two subventral and one dorsal, present but not obvious. Two pairs of genital papillae ventral (vn) to alae, six pairs forming bursal rays, ending distinctly on dorsal side (Rnd), ventral side (Rnv), or margin (Rn) of bursa. Pairs of papillae and phasmids arranged from anterior to posterior as (R1, R2, R3d, (v1, C), v2, (Ph, R4d) (R5d, R6v)), whereby R2 is anterior and closer to R1 than to R3d, R2-R4d are close and regularly spaced, v1 at same level as C, single papillae of v1 further from each other than single papillae of v2, Ph slightly anterior or at same level as R4d, and R5d and R6v sharing same opening. Tail bluntly conical. Bursa peloderan, narrow, such that alae extend less than one-third cloacal body diam. from body, terminating in two posterior points.

#### *Hermaphrodite*

Body very stout. Each genital system arranged from vulva/vagina as uterus, oviduct, and ovotestis. In J4 or very young adults, uterus and oviduct extending ventrally and anteriorly on right of intestine, as typical for diplogastrids, but in most adults gonads are expanded, possibly tangled, and filled with developing brood, such that positions of gonad arms are unclear. Ovotestis totally reflexed, extending dorsally. Dorsal flexure of gonad short, not usually extending to vulva, remaining short even after extension of main gonad branch during J4 and adult stages in which oocytes are arranged in 2-3 rows, often terminating in one large oocyte just proximal to gonad flexure. At proximal end of main gonad branch, oviduct serving as spermatheca and filled with self sperm. Distal to spermatheca-like region, oviduct of young hermaphrodites composed of flattened, rectangular cells. Remaining distal part of oviduct containing developing embryos from an early stage, even as early as J4, before eruption of vulva and moulting to adulthood. Developing embryos and juveniles seemingly arranged haphazardly along body of mature mother. Vaginal glands present but obscure, observed in ventral view. Vagina perpendicular to body surface. Sac-like cells lining vulva anteriorly

and posteriorly with dense, refractive cytoplasm such that sacs appear thickened. Vulva slightly to strongly protuberant in lateral view, pore-like in ventral view. Rectum *ca* 1 anal body diam. long, intestinal-rectal junction surrounded by well developed sphincter muscle. Three rectal glands, two subventral and one dorsal, present. Anus in form of dome-shaped slit, posterior anal lip slightly protuberant. Phasmid conspicuous, located *ca* one-third anal body diam. anterior to anus. Tail thick and conical, *ca* 3-4 anal body diam. in length, with conical terminus.

#### TYPE HOST (CARRIER) AND LOCALITY

The type strain was established from an individual nematode isolated from dung beetles of the tribe Canthonini and from *Onthophagus* (Coleoptera: Scarabaeidae) on a farm near Pretoria, South Africa.

#### TYPE MATERIAL AND STRAIN CULTURE

Holotype male, two paratype males, and six paratype hermaphrodites deposited in the UCRNC, Riverside, CA, USA. Four paratype hermaphrodites deposited in the Natural History Museum Karlsruhe, Karlsruhe, Germany. The type strain is available in living culture from the Department of Evolutionary Biology, MPI for Developmental Biology, Tübingen, Germany and can be provided to other researchers upon request. Attempts to achieve frozen stocks of this species have been unsuccessful.

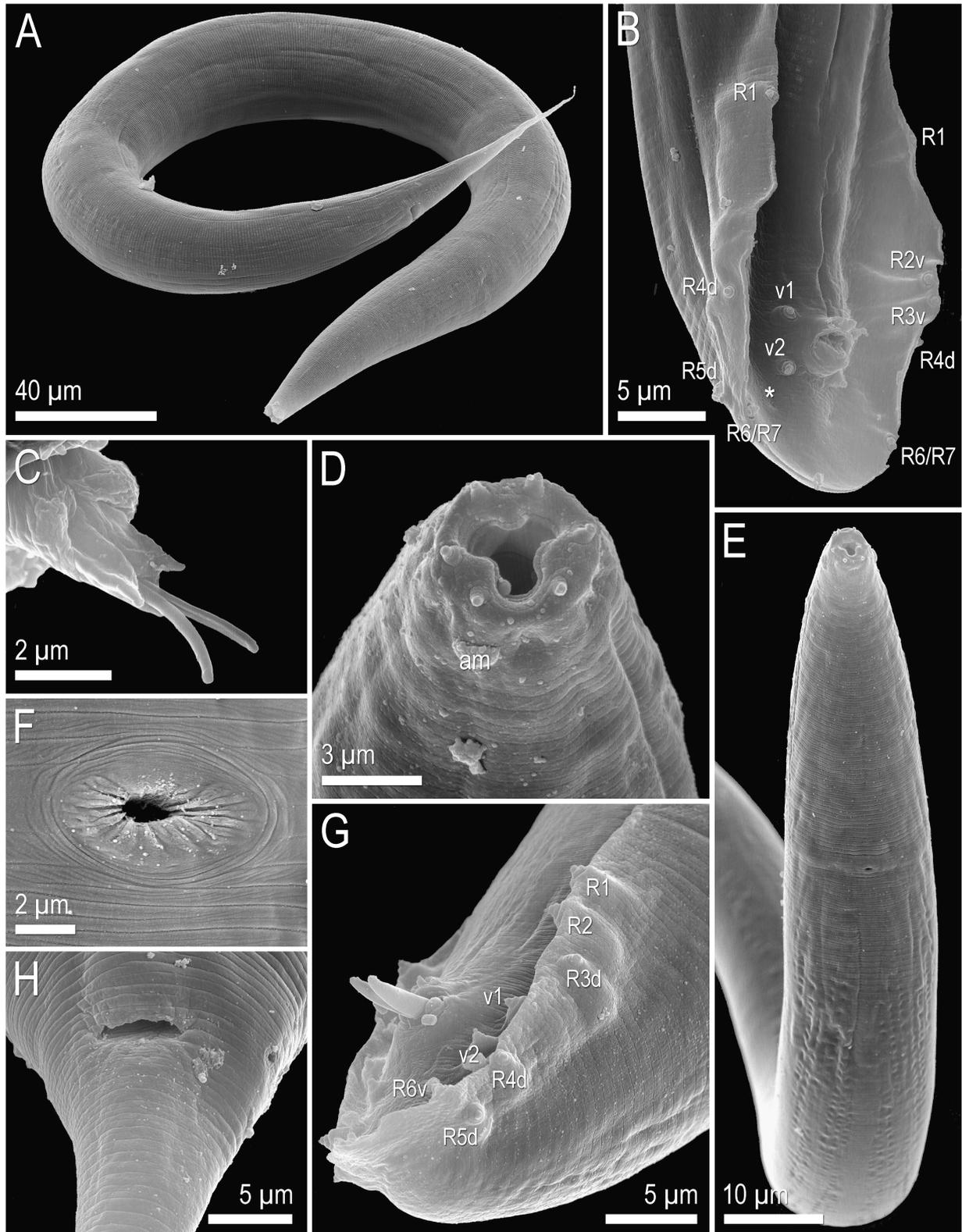
#### OTHER CARRIER AND LOCALITY

In addition to its collection from the type host (carrier) and locality, the species has been isolated from dung beetles of *Scarabaeus* L. 1758 that were collected on Corsica by Prof. H. Schultz-Key.

#### DIAGNOSIS AND RELATIONSHIPS

*Sudhausia crassa* n. gen., n. sp. is diagnosed by a very stout body shape, stout and robust spicules with a long lamina (one-third of spicule length), male genital papillae including only eight pairs and arranged as (R1, R2, R3d, (v1, C), v2, (Ph, R4d) (R5d, R6v)), and the phasmid of the hermaphrodite opening anterior to the anus.

It is distinguished from the type and only other species in the genus as described thereunder.



## Discussion

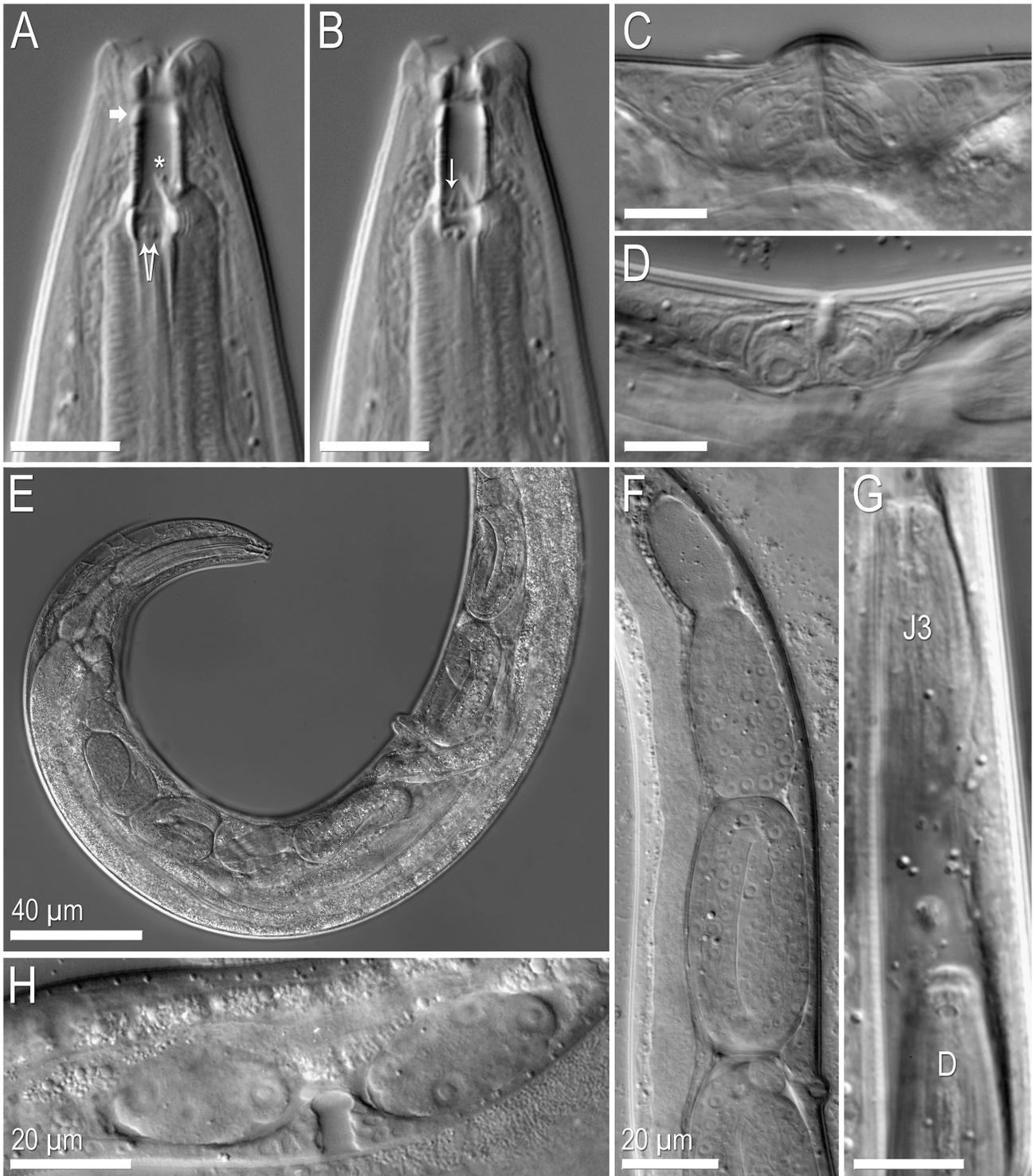
*Sudhausia* n. gen. is described according to a novel combination of traits. Whereas some individual characters, including a tube-shaped gymnostom, subventral stegostomatal armature, and a developed bursa, independently circumscribe other genera of Diplogastridae, their shared presence in *Sudhausia* n. gen. clearly distinguishes the new genus from other lineages in the family. The composition of stomatal traits in particular raises questions about the evolution and possible convergence of the architecture of those structures.

Stomatal characters most clearly diagnose *Sudhausia* n. gen. from other diplogastrids. Almost all sequenced diplogastrid genera characterised by a tube-shaped gymnostom, namely *Demaniella*, *Diplogasteroides*, *Diplogastrellus*, *Pseudodiplogasteroides* and *Rhabditoides*, putatively constitute a monophyletic clade (Mayer *et al.*, 2009; Kiontke & Fitch, 2010; Figs 1, 2), with the possible exclusion of the highly autapomorphic genus *Rhabditolaimus*, formerly of a separate family, Cyliandrocorporidae (Goodey, 1939; Andr assy, 1984). The position of *Sudhausia* n. sp. was variably supported as falling outside of this group, suggesting the possibility that a tube-shaped gymnostom has evolved independently in *Sudhausia* n. gen. and other diplogastrids. The presence of a pair of metastegostomatal denticles, presumably homologous with the subventral tooth and armature of diplogastrids with opposing, movable teeth (*e.g.*, *Koerneria*, *Pristionchus*, *Mononchoides*), clearly sets *Sudhausia* n. gen. apart from other genera with a tube-shaped gymnostom. A phylogeny with broader taxon representation is needed to test whether the stoma of *Sudhausia* n. gen. represents an intermediate state in the loss of teeth or is due to convergent evolution of a tubular stoma. Tracking the loss of predatory feeding structures, and the presumptive advantages they entail (Kiontke & Fitch, 2010), holds important implications for the evolution of feeding strategies.

Male sexual characters are also distinct for *Sudhausia* n. gen., which is in part circumscribed by the presence of a male bursa and which shows divergent arrangements of genital papillae. A bursa has apparently arisen independently in different lineages of Diplogastridae, for example, in both *Neodiplogaster* and *Rhabditolaimus* (Figs 1, 2), where this character is even variable among congeners. In known diplogastrid species with a developed bursa, the arrangement of the nine pairs of papillae and rays is relatively conserved, such that their homologies are reliably discernible (Sudhaus & F urst von Lieven, 2003). In *Sudhausia* n. gen., the positions of rays in particular are atypical for diplogastrids, making homologies difficult to interpret in this genus. The anterior and posterior pairs of papillae closest to the cloacal aperture in *Sudhausia* n. gen. are most likely the homologues of the ventral v2/v3 and v4 papillae *sensu* Sudhaus & F urst von Lieven (2003), respectively. Furthermore, the most anterior ray is probably homologous with v1 in other diplogastrids. It is possible that at least ray R5v in *S. crassa* n. gen., n. sp. and rays R6 and R7 in *S. aristotokia* n. gen., n. sp. are homologous with one or two of the small ventral triplets (v5-v7), respectively, in other diplogastrids. The derived condition of only eight pairs of papillae in *S. crassa* n. gen., n. sp. is apparently due to the loss of the homologue of either R6 or R7 present in *S. aristotokia* n. gen., n. sp. The proclivity to lose one or two pairs of these papillae has also been observed in *Parasitodiplogaster* Poinar, 1979 and *Teratodiplogaster* Kanzaki, Giblin-Davis, Davies, Ye, Center & Thomas, 2009 (Giblin-Davis *et al.*, 2006; Kanzaki *et al.*, 2012b). Identities of the other rays are unclear, and plasticity of rays even among species of *Sudhausia* n. gen. indicates that the rays are likely to be divergent with respect to outgroups.

Unusual developmental phenomena distinguish *Sudhausia* n. gen. from other similarly studied nematode

**Fig. 7.** Scanning electron micrographs of *Sudhausia aristotokia* n. gen., n. sp. and *S. crassa* n. gen., n. sp. A-C: *S. aristotokia* n. gen., n. sp. A: Entire body of mature hermaphrodite; B: Male tail, right subventral view. Nomenclature of genital papillae follows text. Asterisk indicates phasmid; C: Gubernaculum and thin tips of spicules extruded from cloacal opening, right subventral view. D-H: *S. crassa* n. gen., n. sp. D: Lip region of hermaphrodite, oblique *en face* and left subdorsal lateral view. Plugged amphid (am) is also apparent; E: Neck region of hermaphrodite, including excretory pore, ventral view; F: Vulva, ventral view; G: Male tail, left ventral view. Extruded, robust spicule tips also apparent; H: Anus, oblique left subventral view. Left phasmid is also apparent. Abbreviations: R1-R7 = bursal ray papillae; d = dorsal, v = ventral, V1, V2 = ventral papillae.



**Table 1.** Morphometrics of male holotypes (in glycerin) and male and hermaphrodite specimens of *Sudhausia aristotokia* n. gen., n. sp. and *S. crassa* n. gen., n. sp. (temporary water mounts). All measurements are in  $\mu\text{m}$  and in the form: mean  $\pm$  sd (range).

Character	<i>S. aristotokia</i> n. gen., n. sp.			<i>S. crassa</i> n. gen., n. sp.		
	Male		Hermaphrodite	Male		Hermaphrodite
	Holotype	Temporary water mounts	Temporary water mounts	Holotype	Temporary water mounts	Temporary water mounts
n	–	5	10	–	6	10
L	479	485 $\pm$ 61 (412-573)	917 $\pm$ 117 (680-1086)	342	444 $\pm$ 45 (363-485)	652 $\pm$ 56 (527-701)
L'	461	450 $\pm$ 49 (390-519)	827 $\pm$ 111 (604-989)	322	413 $\pm$ 46 (331-458)	583 $\pm$ 57 (458-637)
a	15	11 $\pm$ 1.1 (10-13)	13 $\pm$ 1.6 (10-15)	13	12 $\pm$ 1.0 (11-13)	12 $\pm$ 1.4 (10-14)
a'	14	10.5 $\pm$ 1.0 (9.2-12)	12 $\pm$ 1.4 (9-14)	12	11 $\pm$ 0.9 (10-12)	11 $\pm$ 1.4 (8-13)
b	4.5	3.8 $\pm$ 0.4 (3.5-4.3)	6.3 $\pm$ 0.6 (5.2-7.1)	4.9	4.1 $\pm$ 0.4 (3.5-4.5)	5.4 $\pm$ 0.4 (4.7-5.9)
c	27	15 $\pm$ 3.3 (10.6-19)	10 $\pm$ 0.9 (8.9-12)	16	15 $\pm$ 2 (12-18)	9.5 $\pm$ 1.2 (7.7-12)
c'	1.3	3.5 $\pm$ 0.5 (2.4-4.4)	1.8 $\pm$ 0.7 (1.1-3.0)	1.4	1.4 $\pm$ 0.2 (1.3-1.7)	4.6 $\pm$ 0.5 (4.0-5.2)
V	–	–	58 $\pm$ 4.0 (51-63)	–	–	61 $\pm$ 4.0 (55-69)
Max. body diam.	32	43 $\pm$ 4.9 (38-50)	72 $\pm$ 15 (48-99)	26	37 $\pm$ 5.0 (29-44)	55 $\pm$ 4.5 (48-65)
Lip region diam.	8.0	8.8 $\pm$ 1.0 (7.8-10)	12 $\pm$ 0.7 (11-13)	7.8	7.4 $\pm$ 1.1 (6.2-8.8)	7.9 $\pm$ 0.6 (6.7-8.6)
Stoma width	3.3	4.1 $\pm$ 0.7 (3.0-4.8)	4.7 $\pm$ 0.5 (3.7-5.4)	3.2	4.6 $\pm$ 1.0 (3.6-6.2)	4.4 $\pm$ 0.7 (3.4-5.2)
Stoma height	13	13 $\pm$ 0.8 (12-14)	15 $\pm$ 0.9 (14-17)	11	11 $\pm$ 1.3 (9.4-13)	13 $\pm$ 1.2 (11-15)
Corpus length	64	83 $\pm$ 5.2 (75-89)	97 $\pm$ 4.5 (88-102)	38	60 $\pm$ 4.5 (55-68)	74 $\pm$ 4.0 (66-81)
Corpus/pharynx ratio	60	66 $\pm$ 3.3 (63-71)	67 $\pm$ 2.7 (62-72)	54	59 $\pm$ 1.9 (58-63)	62 $\pm$ 2.3 (59-67)
Median bulb diam.	12	11 $\pm$ 0.9 (10-13)	15 $\pm$ 1.5 (13-18)	10	13 $\pm$ 0.5 (12-13)	16 $\pm$ 0.9 (15-17)

**Fig. 8.** Nomarski micrographs of *Sudhausia aristotokia* n. gen., n. sp. and *S. crassa* n. gen., n. sp. A: Stomatal region of hermaphrodite of *S. aristotokia* n. gen., n. sp., sagittal plane, left ventral view. Short arrow indicates offset anterior part of gymnostom; asterisk indicates dorsal tooth; long arrows indicate pair of telostegostomatal denticles; B: Same individual shown in (A), plane left of sagittal, left lateral view. Arrow indicates one of the pair of left subventral metastegostomatal denticles (both are shown); C: Vagina of *S. aristotokia* n. gen., n. sp., sagittal plane, right lateral view. Vagina is lined anteriorly and posteriorly by series of sac-like cells with a thin cytoplasm and membranous appearance; D: Vagina of *S. crassa* n. gen., n. sp., showing vaginal sac-like cells with a refractive cytoplasm and thickened appearance; E: Mature hermaphrodite of *S. aristotokia* n. gen., n. sp., with a series of developing embryos and juveniles in oviduct; F: Oviduct of hermaphrodite of *S. crassa* n. gen., n. sp. with growing embryos; G: Dauer (D) and directly developing J3 (J3) juveniles within the oviduct of *S. crassa* n. gen., n. sp.; H: J4 of *S. crassa* n. gen., n. sp., gravid with developing embryos, located near developing vulva. (Scale bars = 10  $\mu\text{m}$  unless otherwise indicated.)

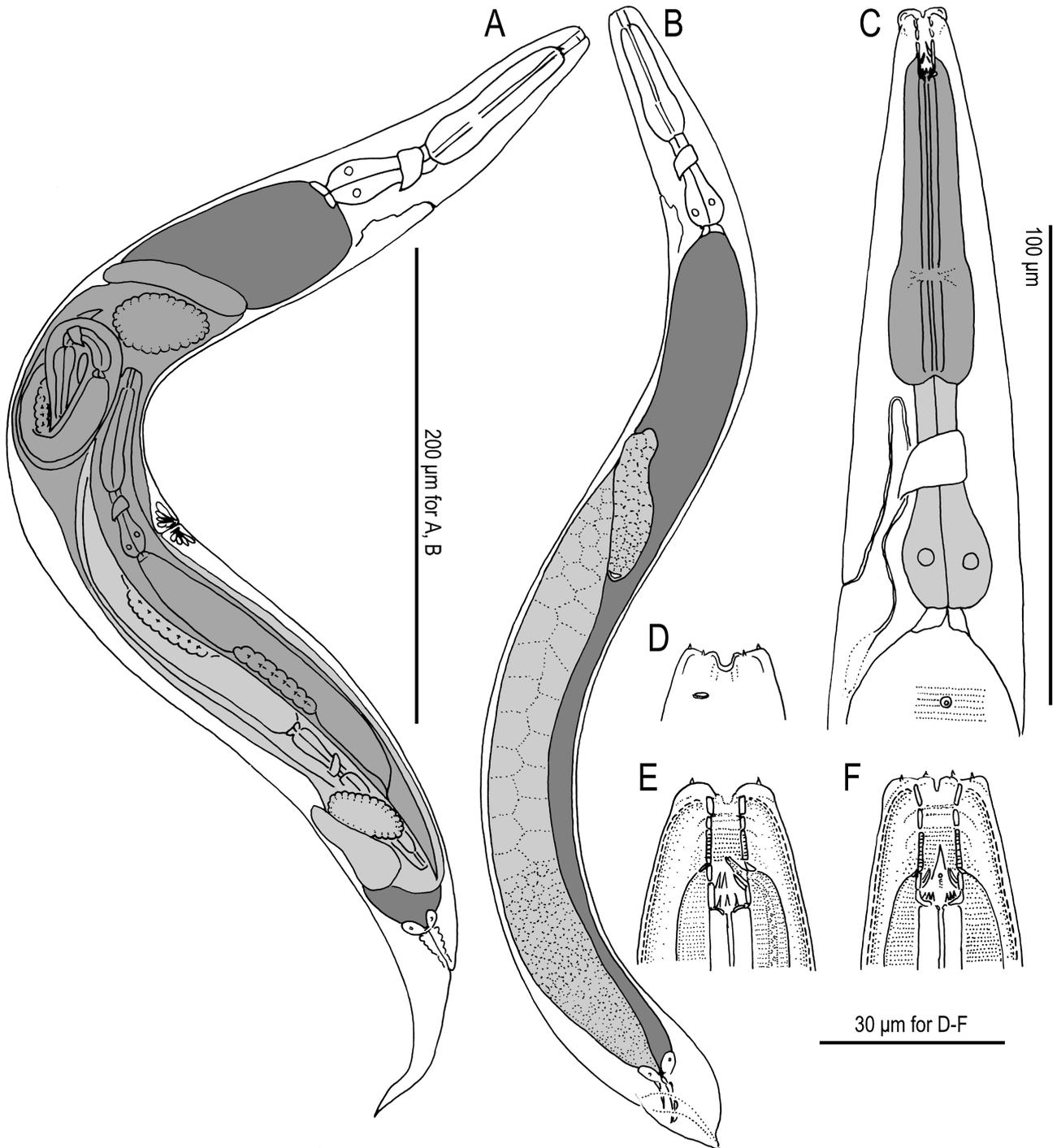
**Table 1.** (Continued.)

Character	<i>S. aristotokia</i> n. gen., n. sp.			<i>S. crassa</i> n. gen., n. sp.		
	Male		Hermaphrodite	Male		Hermaphrodite
	Holotype	Temporary water mounts	Temporary water mounts	Holotype	Temporary water mounts	Temporary water mounts
Basal bulb diam.	12	14 ± 1.3 (13-16)	18 ± 1.8 (16-21)	10	14 ± 1.4 (11-15)	17 ± 1.1 (15-19)
Excretory pore from ant. end	106	97 ± 6.2 (90-103)	112 ± 12 (95-127)	72	83 ± 6.6 (71-90)	105 ± 9.1 (91-115)
Nerve ring from ant. end	88	91 ± 5.0 (87-99)	107 ± 7.8 (96-121)	57	65 ± 2.1 (63-70)	82 ± 6.8 (68-90)
Vulva from ant. end	–	–	479 ± 60 (365-563)	–	–	352 ± 27 (303-385)
Cloacal or anal body diam.	14	20 ± 5.9 (13-27)	26 ± 4.2 (21-35)	15	22 ± 2.5 (19-26)	15 ± 1.7 (13-18)
Tail length	18	35 ± 13 (22-54)	90 ± 8.1 (77-102)	21	31 ± 2.0 (28-33)	69 ± 5.8 (60-79)
Spicule length (curve)	31	30 ± 3.3 (27-35)	–	24	20 ± 0.7 (19-21)	–
Gubernaculum length	10	9.4 ± 1.2 (8.2-11)	–	14	11 ± 0.5 (10-12)	–

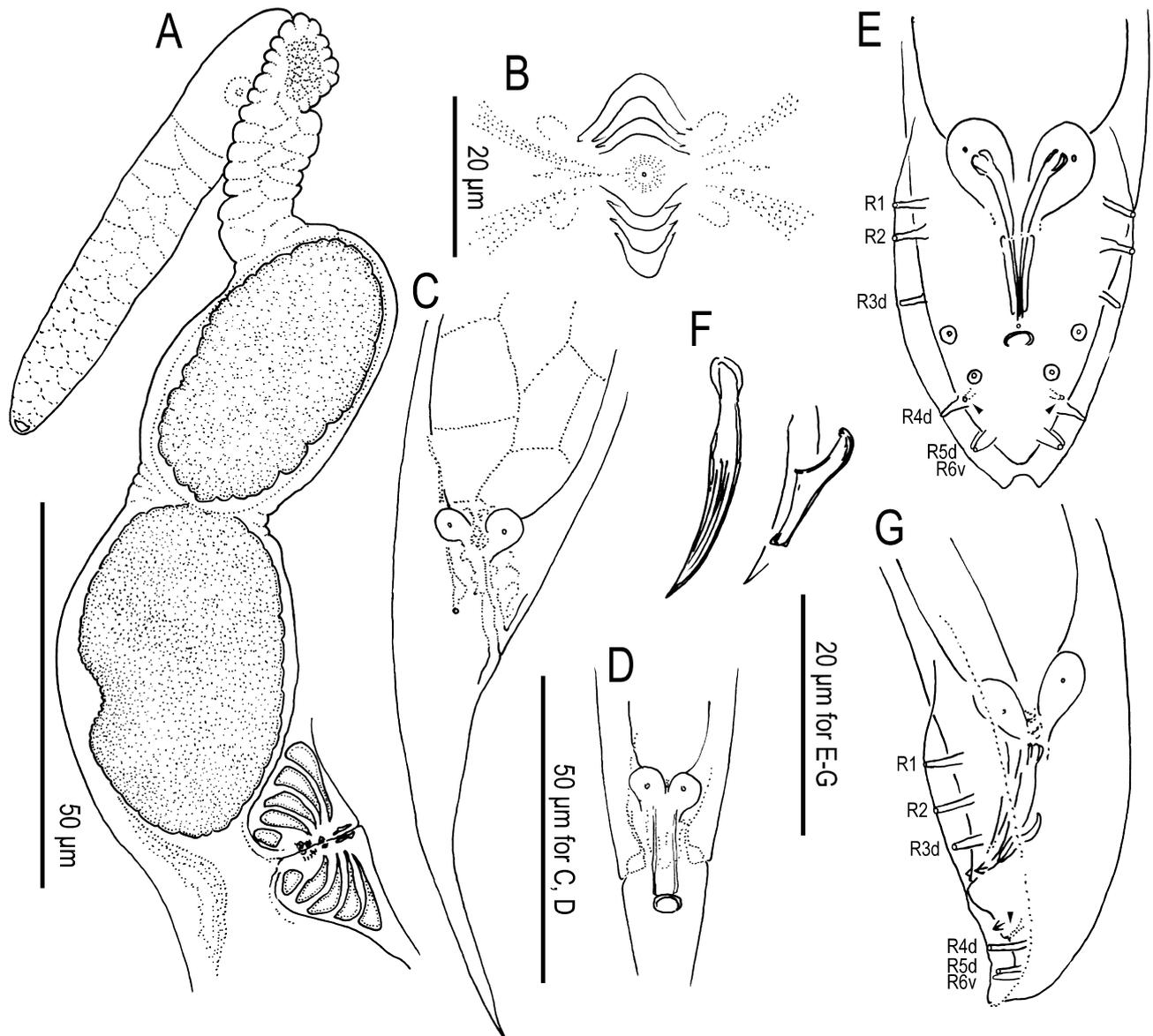
systems. Perhaps most remarkable is the precocious gonad development in *Sudhausia* n. gen., whereby fertilisation and offspring development occur before the mother reaches the adult stage, a phenomenon made possible by self-sufficient (hermaphroditic) reproduction. The two-fold size increase of embryos in *Sudhausia* n. gen. is also unique, to our knowledge, among nematodes and other animals with an eggshell. Although the nematode eggshell is not expected to harden until after being passed through uterine or vaginal sphincters (*e.g.*, Bird *et al.*, 1993), embryonic development as currently perceived proceeds by cell division without an increase in embryo size (Sulston *et al.*, 1983; Schulze & Schierenberg, 2011). Finally, the demonstrated capability for vivipary tentatively distinguishes *Sudhausia* n. gen. from most other diplogastrid species, except for *Koerneria pararmata* (Schneider, 1938) Sudhaus & Fürst von Lieven, 2003 (Fürst von Lieven, 2005) and *Mehdinema alii* Farooqui, 1967 (Luong *et al.*, 1999), although more detailed examination of other diplogastrids exhibiting intra-uterine birth (usually *endotokia matricida*) may reveal further cases of vivipary. However, whereas *endotokia matricida* in particular is commonly a facultative response to a stressful environment (Johnigk & Ehlers, 1999; Chen & Caswell-Chen,

2004), *S. aristotokia* n. gen., n. sp. and *S. crassa* n. gen., n. sp. are constitutively viviparous, even under well-fed laboratory conditions. We hypothesise that the unique vaginal morphology of *Sudhausia* n. gen., namely the anterior and posterior columns of sac-like cells lining the vagina, is correlated with this life-history trait, although presently the function of these structures is unknown.

The isolation of new higher-order taxa such as *Sudhausia* n. gen. from insect associates suggests that this general habitat is still far from being adequately sampled. The collection of new species from beetles in particular promises to reveal further cases of novel trait combinations and expand the known spectrum of morphological and biological diversity in Diplogastridae. The presently unclear relationship of *Sudhausia* n. gen. (+ *Tylopharynx*, *Neodiplogaster* and *Mononchoides*) to other major lineages of the family suggests that ongoing sampling will be necessary to provide greater resolution of deeper relationships among them. The collection of new or understudied taxa from other insect hosts (Herrmann *et al.*, unpubl.; Kanzaki, unpubl.) is likely to help in this regard, and it will be essential for refining the framework needed for ongoing macroevolutionary studies in the group.



**Fig. 9.** Mature adults of *Sudhausia crassa* n. gen., n. sp. A: Hermaphrodite, right lateral view; B: Male, left lateral view; C: Neck region of hermaphrodite, left lateral view; D: Lip region of male, left subdorsal view; E: Stomatal region of hermaphrodite, left lateral view; F: Stomatal region of hermaphrodite, ventral view.



**Fig. 10.** Adults of *Sudhausia crassa* n. gen., n. sp. A: Reproductive tract of young hermaphrodite, right lateral view; B: Vulva, ventral view; C: Tail region of hermaphrodite, left lateral view; D: Anus, ventral view; E: Male tail, ventral view; F: Spicule and gubernaculum, left lateral view; G: Male tail, left lateral view. Abbreviations: R1-R6 = bursal ray papillae; d = dorsal; v = ventral.

## Acknowledgements

The first three authors contributed equally to this work. We thank Clarke Scholtz and his team for their hospitality in South Africa, Keith Philips with the Ghana Insect Project and Jürgen Berger for SEM. We also thank

Hartwig Schulz-Key and Andreas Weller for dung beetle samples and Heike Haussmann for culturing strains of the new species. We thank Walter Sudhaus, Alexander Fürst von Lieven, Karin Kiontke, Einhard Schierenberg and Aldo Zullini for sharing their expertise. Finally, we are thankful to the late Werner Mayer for his molecular biology work early in this project.

## References

- Andrássy, I. (1970). Freilebende Nematoden aus Vietnam. *Opuscula Zoologica Budapest* 12, 105-106.
- Andrássy, I. (1984). *Klasse Nematoda*. Stuttgart, Germany, Gustav Fischer Verlag.
- Baldwin, J.G., Giblin-Davis, R.M., Eddleman, C.D., Williams, D.S., Vida, J.T. & Thomas, W.K. (1997). The buccal capsule of *Aduncospiculum halicti* (Nemata: Diplogastrina): an ultrastructural and molecular phylogenetic study. *Canadian Journal of Zoology* 75, 407-423.
- Bento, G., Ogawa, A. & Sommer, R.J. (2010). Co-option of the hormone-signalling module dafachronic acid-DAF-12 in nematode evolution. *Nature* 466, 494-497.
- Bird, A.F., De Ley, P. & Bird, J. (1993). Morphology, oviposition, and embryogenesis in an Australian population of *Acroboloides nanus*. *Journal of Nematology* 25, 607-615.
- Chen, J.J. & Caswell-Chen, E.P. (2004). Facultative vivipary is a life-history trait in *Caenorhabditis elegans*. *Journal of Nematology* 36, 107-113.
- Cobb, N.A. (1924). *Neodiplogaster tropica* n. g. (?) n. sp. *Journal of Parasitology* 11, 105.
- de Man, J.G. (1876). Onderzoekingen over vrij in de aarde levende Nematoden. *Tijdschrift der Nederlandsche Dierkundige Vereeniging* 2, 78-196, pls. 3-13.
- de Man, J.G. (1912). *Odontopharynx longicaudata* n. g. n. sp. Eine neue Form von Anguilluliden. *Zoologische Jahrbücher, Abteilung für Systematik* 33, 637-642.
- Edgar, R.C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32, 1792-1797.
- Farooqui, M.N. (1967). On a new family Mehdinematidae fam. nov. from *Gryllus domesticus*. *Zoologischer Anzeiger* 178, 322-326.
- Floyd, R., Abebe, E., Papert, A. & Blaxter, M. (2002). Molecular barcodes for soil nematode identification. *Molecular Ecology* 11, 839-850.
- Fuchs, G. (1914). Über Parasiten und andere biologisch an die Borkenkäfer gebundene Nematoden. 85. *Verhandlung der Gesellschaft Deutscher Naturforscher und Ärzte* 2, 688-692.
- Fürst von Lieven, A. (2005). The embryonic moult in diplogastrids (Nematoda) – homology of developmental stages and heterochrony as a prerequisite for morphological diversity. *Zoologischer Anzeiger* 244, 79-91.
- Fürst von Lieven, A. & Sudhaus, W. (2000). Comparative and functional morphology of the buccal cavity of Diplogastrina (Nematoda) and a first outline of the phylogeny of this taxon. *Journal of Zoological Systematics and Evolutionary Research* 38, 37-63.
- Fürst von Lieven, A. & Sudhaus, W. (2008). Description of *Oigolaimella attenuata* n. sp. (Diplogastridae) associated with termites (*Reticulitermes*) and remarks on life cycle, giant spermatozoa, gut-inhabiting flagellates and other associates. *Nematology* 10, 501-523.
- Fürst von Lieven, A., Uni, S., Ueda, K., Barbuto, M. & Bain, O. (2011). *Cutidiplogaster manati* n. gen., n. sp. (Nematoda: Diplogastridae) from skin lesions of a West Indian manatee (Sirenia) from the Okinawa Churaumi Aquarium. *Nematology* 13, 51-59.
- Giblin-Davis, R.M., Ye, W., Kanzaki, N., Williams, D., Morris, K. & Thomas, W.K. (2006). Stomatal ultrastructure, molecular phylogeny, and description of *Parasitodiplogaster laevigata* n. sp. (Nematoda: Diplogastridae), a parasite of fig wasps. *Journal of Nematology* 38, 137-149.
- Goodey, T. (1939). *Cylindrocorpus* nom. nov. for *Cylindrogaster* Goodey, 1927 (Nematoda). *Journal of Helminthology* 17, 149-150.
- Herrmann, M., Mayer, W.E. & Sommer, R.J. (2006). Sex, bugs and Haldane's rule: the nematode genus *Pristionchus* in the United States. *Frontiers in Zoology* 3, 14.
- Hirschmann, H. (1952). Die Nematoden der Wassergrenze mitteleuropäischer Gewässer. *Zoologische Jahrbücher, Abteilung für Systematik* 81, 313-407.
- Hooper, D.J. (1986). Handling, fixing, staining and mounting nematodes. In: Southey, J.F. (Ed.). *Laboratory methods for work with plant and soil nematodes*. London, Her Majesty's Stationery Office, Ministry of Agriculture and Food, pp. 59-80.
- Johnigk, S.-A. & Ehlers, R.-U. (1999). *Endotokia matricida* in hermaphrodites of *Heterorhabditis* spp. and the effect of food supply. *Nematology* 1, 717-726.
- Kanzaki, N., Giblin-Davis, R.M., Davies, K., Ye, W., Center, B.J. & Thomas, W.K. (2009). *Teratodiplogaster fignewmani* gen. nov., sp. nov. (Nematoda: Diplogastridae) from the syconia of *Ficus racemosa* in Australia. *Zoological Science* 26, 569-578.
- Kanzaki, N., Ragsdale, E.J., Herrmann, M., Mayer, W.E., Tanaka, R. & Sommer, R.J. (2012a). *Parapristionchus giblindavisi* n. gen., n. sp. (Rhabditida: Diplogastridae) isolated from stag beetles (Coleoptera: Lucanidae) in Japan. *Nematology* 14, 933-947.
- Kanzaki, N., Giblin-Davis, R.M., Davies, K. & Center, B.J. (2012b). *Teratodiplogaster martini* n. sp. and *Parasitodiplogaster doliostoma* n. sp. (Nematoda: Diplogastridae) from the syconia of *Ficus* species from Africa. *Nematology* 14, 529-546.
- Kanzaki, N., Ragsdale, E.J., Herrmann, M., Röseler, W. & Sommer, R.J. (2013). Two new species of *Pristionchus* (Nematoda: Diplogastridae) support the biogeographic importance of Japan for the evolution of the genus *Pristionchus* and the model system *P. pacificus*. *Zoological Science* 30, in press.
- Kiontke, K. & Fitch, D.H.A. (2010). Phenotypic plasticity: different teeth for different feasts. *Current Biology* 20, R710-R712.
- Körner, H. (1954). Die Nematodenfauna des vergehenden Holzes und ihre Beziehungen zu den Insekten. *Zoologische Jahrbücher, Abteilung für Systematik* 82, 245-353.

- Kreis, H.A. (1932). Beiträge zur Kenntnis pflanzenparasitischer Nematoden. *Zeitschrift für Parasitenkunde* 5, 184-194.
- Luong, L.T., Platzer, E.G., De Ley, P. & Thomas, W.K. (1999). Morphological, molecular, and biological characterization of *Mehdinema alii* (Nematoda: Diplogasterida) from the decorated cricket (*Gryllodes sigillatus*). *Journal of Parasitology* 85, 1053-1064.
- Mayer, W.E., Herrmann, M. & Sommer, R.J. (2007). Phylogeny of the nematode genus *Pristionchus* and implications for biodiversity, biogeography and the evolution of hermaphroditism. *BMC Evolutionary Biology* 7, 104.
- Mayer, W.E., Herrmann, M. & Sommer, R.J. (2009). Molecular phylogeny of beetle associated diplogastrid nematodes suggests host switching rather than nematode-beetle coevolution. *BMC Evolutionary Biology* 9, 212.
- Meyl, A.H. (1960). Freilebende Nematoden. In: Brohmer, P., Ehrmann, P. & Ulmer, G. (Eds). *Die Tierwelt Mitteleuropas: Freilebende Nematoden*. Leipzig, Germany, Quelle & Meyer.
- Micoletzky, H. (1922). Die freilebenden Erd-Nematoden. *Archiv für Naturgeschichte* 87A, 1-650.
- Paramonov, A.A. (1952). Opyt ekologicheskoi klassifikatsii fitonematod. *Trudy Gelmintologicheskoi Laboratorii, Akademia Nauk SSSR (Moskva)* 6, 338-369.
- Poinar Jr, G.O. (1979). *Parasitodiplogaster sycophilon* gen. n., sp. n. (Diplogasteridae: Nematoda), a parasite of *Elisabethiella stuckenbergi* Grandi (Agaonidae: Hymenoptera) in Rhodesia. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen (Ser. C)* 82, 375-381.
- Rae, R., Riebesell, M., Dinkelacker, I., Wang, Q., Herrmann, M., Weller, A.M., Dieterich, C. & Sommer, R.J. (2008). Isolation of naturally associated bacteria of necromenic *Pristionchus* nematodes and fitness consequences. *Journal of Experimental Biology* 211, 1927-1936.
- Rahm, G. (1928). Alguns nematodes parasitas e semiparasitas das plantas culturães do Brasil. *Archivos do Instituto de Biológico de Defesa Agricola e Animal (São Paolo)* 1, 239-251.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61, 539-542.
- Schneider, W. (1938). *Diplogaster pararmatus* n. n. nebst Bemerkungen über einige andere *Diplogaster*-Arten. *Zoologischer Anzeiger* 121, 37-43.
- Schulze, J. & Schierenberg, E. (2011). Evolution of embryonic development in nematodes. *EvoDevo* 2, 18.
- Sommer, R.J. & McGaughran, A. (2013). The nematode *Pristionchus pacificus* as a model system for integrative studies in evolutionary biology. *Molecular Ecology* 22, 2380-2393.
- Stamatakis, A. (2006). RAXML-VI-HPC: maximum likelihood-based analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688-2690.
- Steiner, G. (1914). Freilebende Nematoden aus der Schweiz. 1.+2. Teil. *Archiv für Hydrobiologie und Planktonkunde* 9, 259-276, 420-438.
- Sudhaus, W. & Fürst von Lieven, A. (2003). A phylogenetic classification and catalogue of the Diplogastridae (Secernentea, Nematoda). *Journal of Nematode Morphology and Systematics* 6, 43-90.
- Sulston, J.E., Schierenberg, E., White, J.G. & Thomson, J.N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Developmental Biology* 100, 64-119.
- Susoy, V. & Herrmann, M. (2012). Validation of *Rhabditolaimus* Fuchs, 1914 (Nematoda: Diplogastridae) supported by integrative taxonomic evidence. *Nematology* 14, 595-604.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28, 2731-2739.
- van Megen, H., van den Elsen, S., Holterman, M., Karszen, G., Mooyman, P., Bongers, T., Holovachov, O., Bakker, J. & Helder, J. (2009). A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences. *Nematology* 11, 927-950.