

Nematodes from the Gulf of California. Part 3. Three new species of the genus *Diplopetoides* Gerlach, 1962 (Nematoda: Diplopetoididae) with overviews of the genera *Diplopettis* Gerlach, 1962 and *Diplopettula* Gerlach, 1950

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Summary. Three new species, *Diplopetoides axayacatl* sp. n., *D. santaclarae* sp. n. and *D. paramastigia* sp. n., are described using light microscopy. All three species are morphometrically close to *D. mastigia* but the former differs in having a hook-shaped amphid, while the latter two can be distinguished from *D. mastigia* by having a gubernaculum with apophysis, and from each other by having, respectively, a striated cuticle (*versus* smooth) and a punctated interamphidial shield (*versus* crenate). The status and composition of *Diplopetoides* is discussed and compared with those of *Diplopettis* and *Diplopettula*. The following species are transferred to different genera: *Diplopettula curta* is placed in *Pararaeolaimus*; *Diplopettis bathmanni* is placed in *Diplopettula*; *Diplopettula pulcher* and *D. sundensis* are transferred to *Diplopetoides*. Three species of *Diplopettula* are considered *species incertae sedis*: *D. botula*, *D. striata* and *D. lucanica*.

Key words: Baja California, *Diplopettis*, *Diplopetoides axayacatl* sp. n., *Diplopetoides paramastigia* sp. n., *Diplopetoides santaclarae* sp. n., *Diplopettula*, morphology, morphometrics, SEM, taxonomy.

The genus *Diplopetoides* Gerlach, 1962 was proposed for the species *Diplopettis ornatus* Gerlach, 1950 with the main diagnostic characters: "complicated cuticular structure and a ring-shaped cephalic capsule". The genus was originally placed in the family Diplopettidae Filipjev, 1918. The original description was based on one male from the North Sea (Gerlach, 1950) and was further supplemented by Timm (1961), who found females in the Bay of Bengal. Lorenzen (1981) had some new material of *Diplopetoides ornatus* at hand, and amended the description of this species with important data about the structure of the pharynx and female reproductive system. The pharynx was stated to have extremely thin walls without muscles, at least in the middle section, expanding to form a bulb at the base. Females were found to have two antidromously reflexed ovaries (not known before for *Diplopetoides*). At the same time Lorenzen (1981) proposed a new family Aegialoalaimidae Lorenzen, 1981 for the genera

Aegialoalaimus de Man, 1907, *Cyartonema* Cobb, 1920, *Diplopetoides* and *Paraterschellingia* Kreis in Stekhoven, 1935 with the following diagnosis: "the pharynx has extremely thin walls and no muscles at least in the middle section and, in part also in the anterior section, and at the end it swells out to form a bulb". Other characters listed are: annulated cuticle, only cephalic sensilla are visible, large amphids are round or inverted V-shaped, stoma very narrow and toothless, females with two antidromously reflexed ovaries and the males with one or two testes. Furthermore, he placed the new family Aegialoalaimidae in the order Chromadorida, whereas the family Diplopettidae was retained in the order Monhysterida.

Two more species were added to the genus *Diplopetoides* by Tchesunov (1990): *D. mastigia* Tchesunov, 1990 was described from one female and two males; *D. anatolii* (Voronov, 1982) Tchesunov, 1990, was transferred from the genus *Diplopettula* Gerlach, 1950. The latter species was

originally described on the basis of three females and two males, and Tchesunov (1990) supplemented the original description with new data on one female and one male; both populations originated from the White Sea. At the same time, Tchesunov split the family Aegialoalaimidae into three separate families, adding Cyartonematidae Tchesunov, 1990 for *Cyartonema* and *Paraterschellingia* and Diplopeltoididae Tchesunov, 1990 for *Diplopeltoides*.

Tchesunov's paper was apparently not available to Jensen (1991), who described what he assumed to be "the second" species of the genus, *Diplopeltoides linkei* Jensen, 1991 and compared it only with *D. ornatus*. Another species was added to the genus *Diplopeltoides* by Vincx & Goubault (1992), who transferred *Diplopeltis onustus* Wieser, 1956 based on the presence of a prominent "cephalic capsule" (= cuticular plate) in both *D. onustus* and in *D. ornatus*. They did not cite either Tchesunov (1990) or Jensen (1991), and were unaware that *D. onustus* was synonymised with *Diplopeltula incisa* (Southern, 1914) Gerlach, 1962 by Voronov (1982) as discussed below. Furthermore, Vincx & Gorbault (1992) did not agree with Lorenzen (1981), who transferred four species from *Diplopeltula* to *Diplopeltis*: *D. incisus* (Southern, 1914) Gerlach, 1950, *D. indicus* (Gerlach, 1962) Lorenzen, 1981, *D. intermedius* (Gerlach, 1954) Lorenzen, 1981 and *D. onustus* Wieser, 1956. Nor did they comment on the systematic position of the genus *Diplopeltoides*, other than considering it to be closely related to *Diplopeltula* and retaining it within the family Diplopeltidae (Vincx & Goubault, 1992) as opposed to Lorenzen's (1981) earlier placement of the genus in Aegialoalaimidae.

Although the genus *Diplopeltoides* is currently placed in the order Plectida (De Ley & Blaxter, 2002, 2004; Holovachov, 2006; Tchesunov, 2006), uncertainty about its phylogenetic affinities necessitates a comparison of our new species with members of the genus *Diplopeltula* and other Diplopeltidae. Major diagnostic characters of *Diplopeltoides* and Diplopeltoididae include the morphology of pharynx and female reproductive system, data that are missing from many older species descriptions or described in males only. In this paper we primarily address improvements to our knowledge of morphological features in the genus as well as problems with species validity and composition in *Diplopeltoides*. No molecular diagnostics or phylogenetics could as yet be performed because of the very limited numbers of

specimens recovered from our samples, and the use in some cases of fixation methods inappropriate for reliable DNA sequencing. Molecular analyses will clearly be needed to resolve the position of Diplopeltoididae, which will require suitable material not just of *Diplopeltoides* but also of the other genera discussed below.

MATERIAL AND METHODS

Intertidal sediment samples were collected in several locations in the Gulf of California, Mexico. The first survey was conducted during November 2002 at several locations including Punta Estrella (30°53' N latitude and 114°42' W longitude) and Don Abel (30°11' N latitude and 114°54' W longitude), located southwest of the Colorado River Delta. These samples were immediately fixed in 95% ethanol *in toto* and decanted and sieved in DI water in 2007.

A second set of samples was collected in November 2004 from El Golfo de Santa Clara (31°41' N latitude and 114°30' W longitude) at the eastern edge of the Colorado River Delta in the swash zone as described in Mundo *et al.* (2007). Three additional samples were collected at the beach (31°40' N latitude and 114°30' W longitude), directly across from Hotel 'Las Conchas' in El Golfo de Santa Clara. They were immediately fixed in hot 4% formaldehyde solution and brought back to the Department of Nematology at the University of California – Riverside where they were decanted and sieved six times after suspension in a Ludox solution with specific gravity of 1.15 (Mundo *et al.*, 2007). The sampling sites were revisited in May 2006 (based on GPS readings) for collection of a second series of samples where the nematodes were immediately decanted, sieved and preserved by replacing the salt water with DESS (Yoder *et al.*, 2006).

A separate set of samples was collected in El Tornillal Beach, situated 25 km south from Santa Clara (31°33' N latitude and 114°17' W longitude), in September 2007 and March 2008. Samples were immediately fixed on site with 5% formaldehyde and brought to the laboratory facilities of CICESE, Ensenada, where they were decanted and sieved four times after suspension in a Ludox solution with specific gravity of 1.15 (De Jonge & Bouwman, 1977).

Preserved specimens were transferred to pure glycerin using Seinhorst's (1959) rapid method as modified by De Grisse (1969). Permanent mounts on glass slides were prepared using the paraffin wax ring method. An Olympus BX51 was used for most light microscope observations. Line drawings

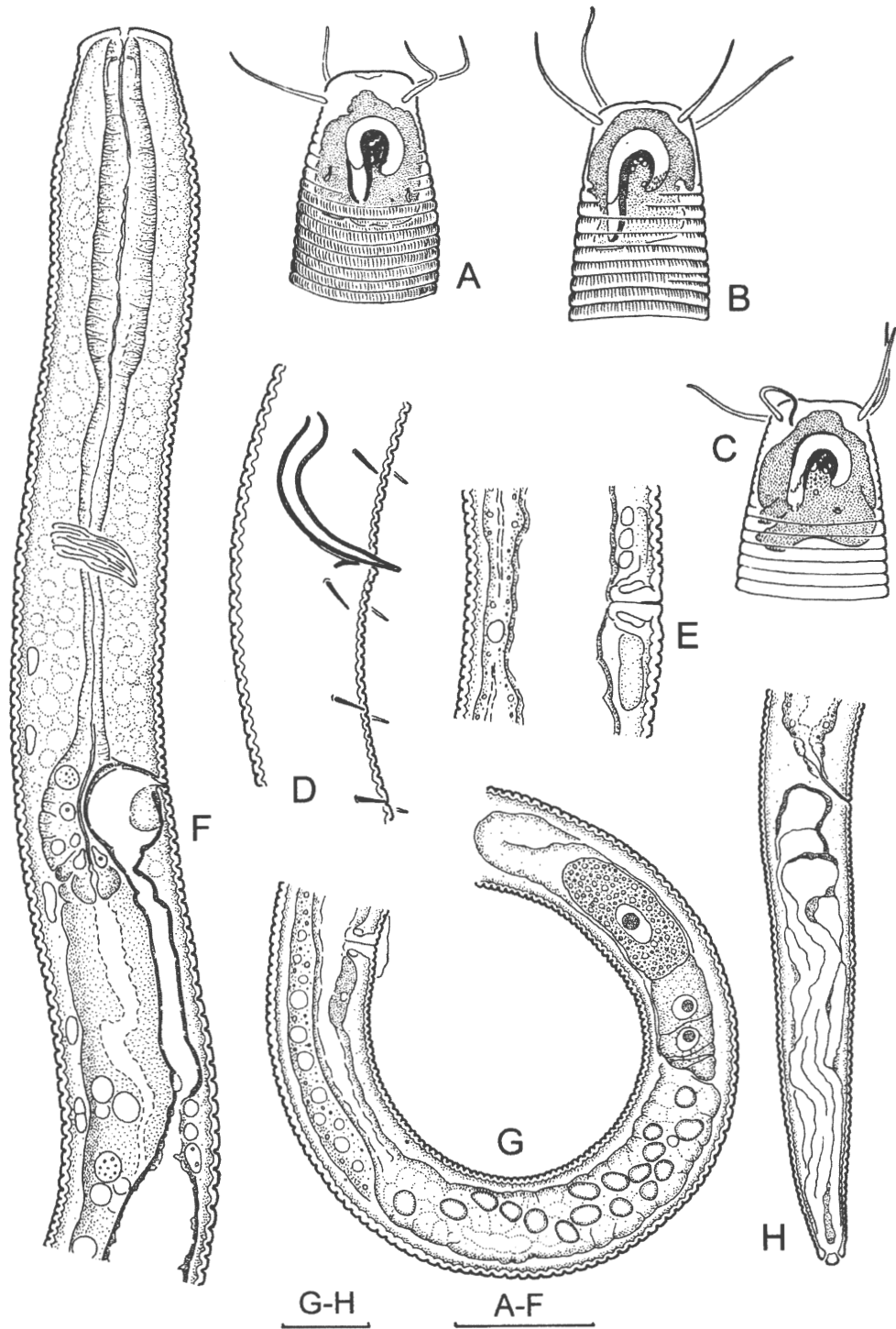


Fig. 1. *Diplopettoides axayacatli* sp. n. A, C, E-H: Female, B, D: Male. A-C: Anterior end, surface view (ventral to right); D: Cloacal region; E: Vulva; F: Pharyngeal region; G: Vulval region and posterior ovary. H: Female tail. (Scale bars: A-H = 20 μm.).

of male caudal regions depict setae filled in with black when they are located on the side of the body facing the reader, while setae left open are located on the opposite side. For electron microscopy, several adults of *D. paramastigia* sp. n.

were postfixed in 1% osmium tetroxide (OsO₄) and transferred to pure acetone through an acetone/distilled water series. The specimens were critical point dried in liquid CO₂, mounted on a stub, gold-plated under vacuum in a sputter and

examined in a Philips XL30 FEG Scanning Electron Microscope (SEM).

TAXONOMY OF THE GENUS *DIPLOPELTOIDES* GERLACH, 1962

Apart from the three new species described below, only the following species are considered here as belonging to the genus *Diplopetloides*: *D. anatolii*, *D. linkei*, *D. mastigia*, *D. ornatus*, *D. nudus* (Gerlach, 1956) Tchesunov, 2006, *D. pulcher* (Vincx & Gourbault, 1992) **comb. n.** and *D. sundensis* (Jensen, 1978) **comb. n.** These species are united by the morphology of pharynx (wide cylindrical muscularised corpus and narrow non-muscular isthmus with small glandular basal bulb/swelling) and female reproductive system (ovaries reflexed antidromously).

It is not impossible that *D. ornatus* and *D. linkei* are in fact the same species: the only apparent feature distinguishing them from each other is gubernaculum shape. Both descriptions were based on a single male. However, Gerlach did not preserve any type material prior to 1964, and the male of *D. linkei* was actually lost “prior to some detailed observations of the gonad system” (Jensen, 1991). Therefore, it cannot be verified at this point whether the stated differences only reflect intraspecific variability or not.

D. pulcher was described from one male and one female in the genus *Diplopettula* (Vincx & Gourbault, 1992), and the description states that ovaries are reflexed, like in *Diplopetloides*. The pharynx in this species does not show the distinct corpus-isthmus constriction but narrows gradually in the middle like in some of our specimens.

D. nudus was described both from females and males in the genus *Diplopettula* (Gerlach, 1956); its description states that the pharynx is “relatively weakly developed, its posterior end cannot be clearly seen with certainty in the male”. A female specimen from Chile identified as *D. aff. nuda* had reflexed ovaries (Lorenzen, 1981), while another female, described under the combination “*Diplopetloides aff. nudus* (Gerlach, 1956)” also had reflexed ovaries and a typical “diplopetloid” pharynx (Tchesunov, 2006). We concur with Tchesunov (1990, 2006) in assigning this species to the genus *Diplopetloides*.

The single known male of *D. sundensis* differs from every other species of *Diplopettula* and *Diplopetloides* for which males are known in the presence of two precloacal supplements, and the original description states that the pharynx is “dilated in the head region, narrow at the neck and posteriorly gradually enlarged, without

forming a bulb” also suggesting that *D. sundensis* belongs in fact to the genus *Diplopetloides*.

The following morphological characters are described here for the first time in species of the genus *Diplopetloides*: i) regular increasing and decreasing of width of the annules of the cuticle along the body; ii) pharyngeal gland orifices are at the same level in the anterior part of the pharynx; iii) oral opening is triangular (at least in *Diplopetloides santaclarae* sp. n.); iv) caudal gland opening *via* three separate outlets. First three characters confirm close similarity between *Diplopetloides* and Ceramonematidae.

Diplopetloides axayacatl sp. n. (Fig. 1)

Measurements. See Table 1.

Adult. Body cylindrical, posteriorly tapering in the tail region, straight or curved upon fixation. Cuticle coarsely annulated along entire body, except for smooth anterior end and terminal part of the tail; annules 2.2–2.5 μm wide at midbody region; longitudinal striation is present on all annules. Annules unequal in width; annule width increasing gradually from first postlabial annule to annule number 76–89 in female or 80 in male, which is followed by a narrower one located posterior to the pharyngo-intestinal junction; then annule width increases again up to midbody region and decreases gradually with narrowest annule located at anterior third of tail length, followed posteriorly by one much wider annule; width of posteriormost caudal annules gradually decreases towards tail tip. Somatic pores and lateral alae absent. Somatic setae in male present only on tail (see below). Labial region truncate conoid; annulation of the anteriormost part of the body is very weak. Cuticular plate underlying cephalic cuticle around amphid, reaching as far as 19.5–20.0 μm from the anterior end of the body; cuticular plates connected with each other on ventral and on dorsal sides. Inner and outer labial sensilla not seen. Cephalic sensilla setiform, 15.0–20.0 μm long, 3.0–5.0 μm from anterior end. Amphids similar in shape and size between sexes: amphidial fovea an inverted hook-shape with its dorsal branch almost straight and ventral branch rounded; anterior margin of *fovea amphidialis* 4.5–7.0 μm from anterior end. Wide space between amphidial branches (amphidial shield) ornamented with crenate sclerotised edge. Stoma very small, its lining is uniform with the lining of the pharynx. Pharynx distinctly subdivided into anterior corpus and posterior postcorpus; corpus cylindrical or slightly fusiform, muscular; postcorpus consists of

anterior narrow non-muscular isthmus and pear-shaped glandular basal swelling. Pharyngeal gland nuclei indistinct, orifices of three pharyngeal glands are present at the anterior part of the pharynx. Pharyngeal lumen uniform in thickness, tubes and valve-like structures absent. Nerve ring surrounding isthmus at three-fifths the length of pharynx (NR = 60.6%). Secretory-excretory system present; excretory pore located along the ventral body line opposite to the basal bulb; excretory duct very short, leading from pore to ampulla; renette cell elongate-ovoid, its body adjacent and ventral to anterior part of intestine. Tail subcylindrical. Caudal glands opening *via* three separate openings, spinneret absent.

Female. Reproductive system didelphic, amphidelphic, reflexed; ovary branches symmetrical. Anterior ovary situated to right of intestine; posterior ovary situated to left of intestine. Vulva a transverse slit immediately posterior to midbody. Vagina straight, one third of the vulval body diameter, with thick walls and well developed sphincter muscle at its proximal part; *pars refringens vaginae* absent. Intrauterine egg not seen; sperm is present in the uterus. Rectum short, 0.7-0.8 of the corresponding body diameter long.

Male. Reproductive system diorchic, both testes outstretched. Spicules paired and symmetrical, 31 μm (right spicule) and 32 μm (left spicule) long along arc, strongly curved, with rounded manubrium and conoid shaft. Gubernaculum plate-like. One (subventral) pair of precloacal and nine (subventral) pairs of caudal setae.

Diagnosis. *D. axayacatli* sp. n. is particularly characterised by its large body (1.45-1.82 mm), cuticle with longitudinal striation, cuticular plate underlying amphids, inverted hook-shaped amphids with sclerotised amphideal plate, long (15-20 μm) cephalic setae and posterior position of excretory pore.

Relationships. *D. axayacatli* sp. n. is most similar to *D. mastigia* in body size (1.45-1.82 mm vs 1.54-2.48 mm in *D. mastigia*), and broad asymmetrical amphid with wide amphideal shield that is sclerotised. However, the new species differs from *D. mastigia* in the presence of longitudinal striation of cuticle (vs not striated in *D. mastigia*), cuticular plate (vs absent in *D. mastigia*), shape of the amphid (hook-shaped vs loop-shaped in *D. mastigia*), longer cephalic setae (15-20 μm vs 6.5-8.5 μm in *D. mastigia*). *D. axayacatli* sp. n. shows similarities to poorly known *Diplopeltula botula* in body size (1.45-1.82 mm vs 1.66 in *D. botula*) and long cephalic setae (15-20 μm vs 17 μm in *D. botula*), but is clearly distinguished in shape of

amphid (wide hook-shaped vs narrow loop-shaped in *D. botula*), presence of cuticular plate and ornamented amphideal shield (vs absent in *D. botula*), position of excretory pore (EP = 89-91% vs EP ca. 60% in *D. botula*). Other characters separating the new species from other members of the genus *Diplopetooides* and *Diplopeltula botula*, *D. striata* and *D. lucanica* are summarised in Table 2. Compared to the two other new species proposed in this paper, *D. axayacatli* is easily distinguished by the presence of a cuticular plate and absence of a gubernacular apophysis (*versus* the reverse).

Type locality. Mexico, Gulf of California, Santa Clara, samples taken in 2006 (female holotype).

Other locality. Mexico, Gulf of California, Don Abel, samples taken in 2002 (one female and one male).

Type material. Holotype female, one female and one male paratypes deposited in the University of California Riverside Nematode Collection, CA, USA.

Ethymology. The specific epithet acknowledges Dr. Axayácatl Rocha-Olivares for his participation in the collaborative research of the nematodes from the Gulf of California.

Diplopetooides santaclarae sp. n. (Figs. 2 & 3)

Measurements. See Table 1.

Adult. Body cylindrical, posteriorly tapering in the tail region, straight or curved upon fixation. Cuticle coarsely annulated along entire body, except for smooth anterior end and terminal part of the tail; annules 3.0 μm wide at midbody region; longitudinal striation is present on all annules. Annules unequal in width, similar to *D. axayacatli*. Somatic pores and lateral alae absent. Somatic setae in male present only on tail (see below). Labial region rounded. Cuticular plate around amphid absent. Inner labial sensilla not seen; outer labial sensilla papilliform, located on the outer edge of the labial region, just in front of cephalic sensilla, visible only under SEM. Cephalic sensilla setiform, 16.0-19.0 μm long, 3.0-5.0 μm from anterior end. Amphids similar in shape and size between sexes: amphidial fovea an inverted loop-shaped with both branches straight; anterior margin of fovea *amphidialis* 4.0-5.0 μm from anterior end. Narrow space between amphidial branches (amphidial shield) ornamented with crenate edges. Oral opening triangular. Stoma very small, its lining is uniform with the lining of the pharynx. Pharynx distinctly subdivided into anterior corpus and posterior postcorpus; corpus

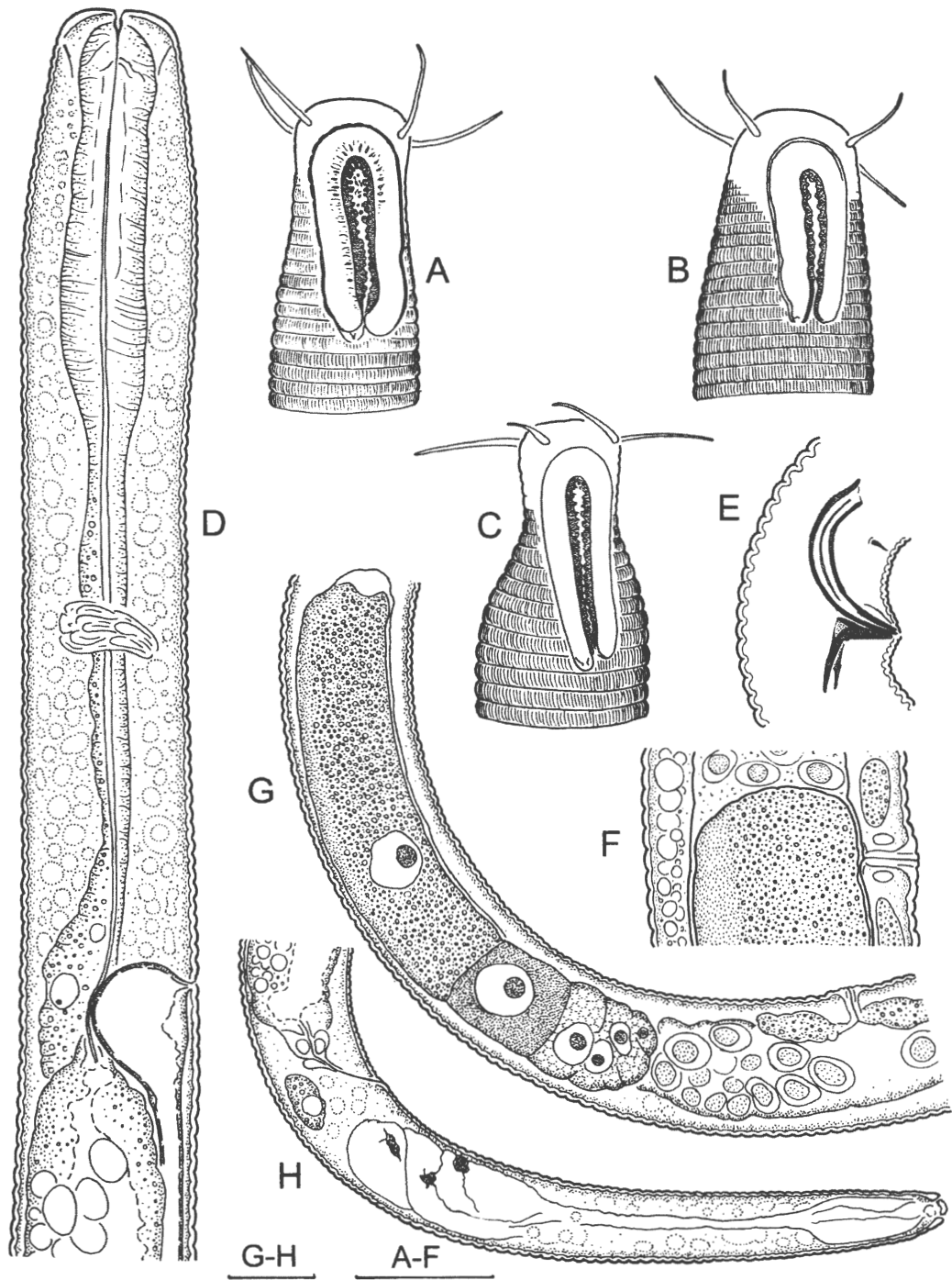


Fig. 2. *Diplopetoides santaclarae* sp. n. A, E: Male, B-D, F-H: Female. A-C: Anterior end, surface view (ventral to right); D: Pharyngeal region; E: Cloacal region; F: Vulva; G: Vulval region and anterior ovary. H: Female tail. (Scale bars: A-H = 20 μ m.).

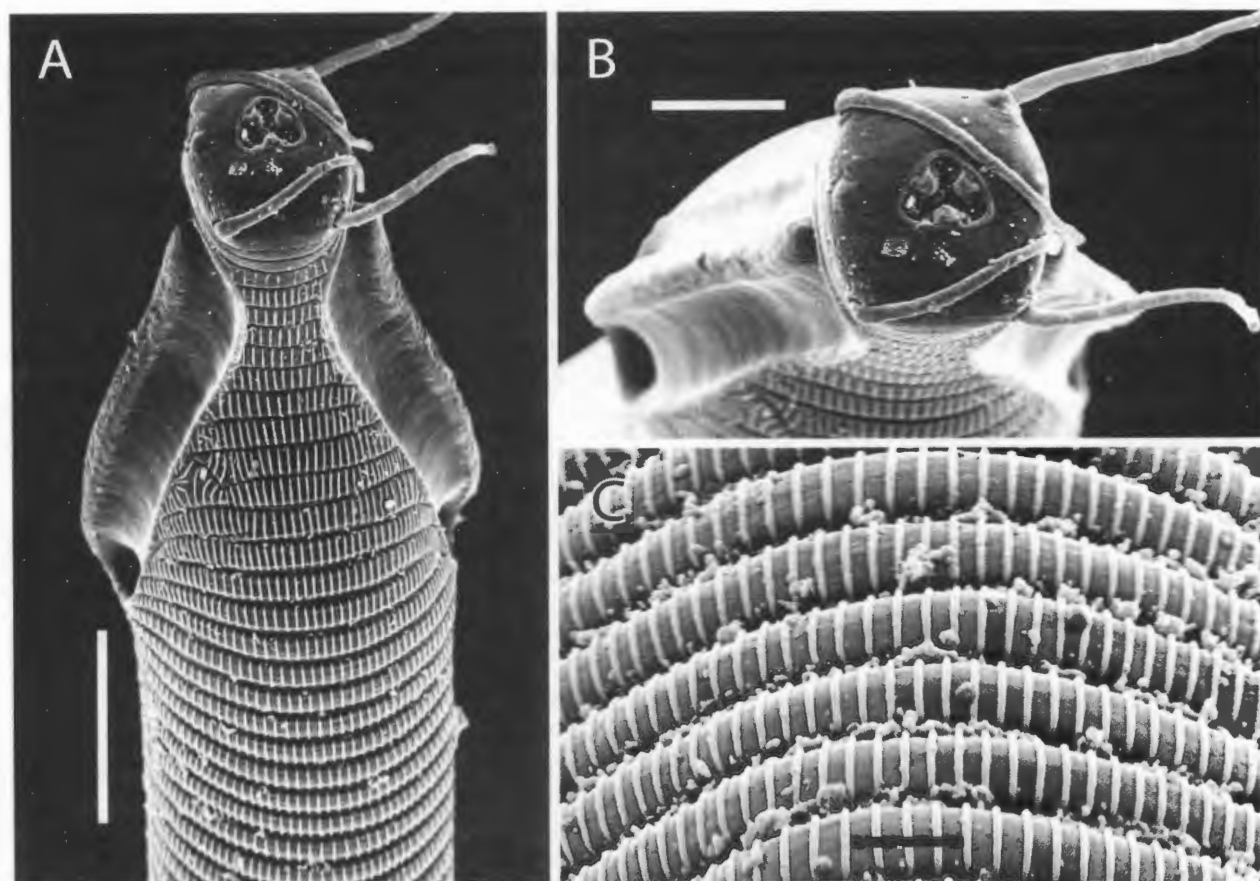


Fig. 3. SEM of *Diplopetoides santaclarae* sp. n. A-B: Labial region, ventral view; C: Cuticle. (Scale bars: A = 20 µm; B = 10 µm; C = 5 µm.).

38-61 µm long, cylindrical or slightly fusiform, muscular; postcorpus 71-104 µm long, consists of anterior narrow non-muscular isthmus and pear-shaped glandular basal swelling. Pharyngeal gland nuclei indistinct, orifices of three pharyngeal glands are present at the anterior part of the pharynx. Pharyngeal lumen uniform in thickness, tubes and valve-like structures absent. Nerve ring surrounding isthmus at approximately three-fifths the length of pharynx (NR = 58-59%). Secretory-excretory system present; excretory pore located along the ventral body line opposite to the basal bulb; excretory duct very short, leading from pore to ampulla; renette cell elongate-ovoid, its body adjacent and ventral to anterior part of intestine. Tail subcylindrical. Caudal glands opening via three separate openings, spinneret absent.

Female. Reproductive system didelphic, amphidelphic, reflexed; ovary branches symmetrical. Anterior ovary situated to right of intestine; posterior ovary situated to left of intestine. Vulva a transverse slit immediately posterior to midbody. Vagina straight, one fifth to one third of the vulval body diameter, with thick

walls and well developed sphincter muscle at its proximal part; *pars refringens vaginae* absent. Intrauterine egg 105x20 µm; sperm is present in the uterus. Rectum short, 1.0-1.2 times as long as the corresponding body diameter long.

Male. Reproductive system diorchic, both testes outstretched. Spicules paired and symmetrical, strongly arcuate, with subcylindrical shaft. Gubernaculum platelike with distinct caudal apophysis. Two (subventral) pairs of precloacal and five (subventral) pairs of caudal setae.

Diagnosis. *D. santaclarae* sp. n. is particularly characterised by large body (1.62-2.04 mm), cuticle with longitudinal striation, no cuticular plate, narrow loop-shaped amphids with sclerotised amphideal plate, long (17.5-19 µm) cephalic setae and posterior position of excretory pore.

Relationships. *D. santaclarae* sp. n. is most similar to *D. mastigia* in its body size (1.62-2.04 mm vs 1.54-2.48 mm in *D. mastigia*), and sclerotised amphideal shield. However, the new species differs from *D. mastigia* in the presence of longitudinal striation of cuticle (vs not striated in

D. mastigia), longer cephalic setae (17.5-19 μm vs 6.5-8.5 μm in *D. mastigia*) and presence of gubernacular apophysis (vs absent in *D. mastigia*). *D. santaclarae* sp. n. displays similarities to *D. anatolii*, *D. nudus* and *Diplopeltula lucanica* in having relatively narrow amphids. It differs from all three species in its larger body size, striated cuticle and other characters listed in Table 2. Compared to the two other new species proposed in this paper, *D. santaclarae* differs from *D. axayacatli* in having amphids with symmetrical branches (versus hook-shaped amphids), in absence of a cuticular plate (versus presence) and in presence of the apophysis (versus absence). *D. santaclarae* differs from *D. paramastigia* in having a striated cuticle (versus smooth) and a shorter rectum (14-20 μm versus 25-28 μm).

Type locality. Mexico, Gulf of California, Santa Clara, El Tornillal, samples taken in 2008 (holotype, two females and one male paratypes).

Type material. Holotype female, two females and one male paratypes deposited in the University of California Riverside Nematode Collection, CA, USA.

Ethymology. The specific epithet is a derivative from the name of the location, Santa Clara, where new species was found.

Diplopeltoides paramastigia sp. n. (Fig. 4)

Measurements. See Table 1.

Adult. Body cylindrical, posteriorly tapering in the tail region, straight or curved upon fixation. Cuticle coarsely annulated along entire body, except for smooth anterior end and terminal part of the tail; annules 1.8-2.2 μm wide at midbody region, annules smooth (no longitudinal striation). Annules unequal in width, similar to *D. axayacatli*. Somatic pores and lateral alae absent. Somatic setae in male present only on tail (see below). Labial region truncate; annulation of the anteriormost part of the body is very weak. Cuticular plate absent around amphid. Inner and outer labial sensilla not seen. Cephalic sensilla setiform, 12.0-18.0 μm long, 2.5-4.5 μm from anterior end. Amphids similar in shape and size between sexes: amphidial fovea an inverted loop-shape with both branches straight; anterior margin of fovea amphidialis 2.5-5.5 μm from anterior end. Wide space between amphidial branches (amphidial shield) ornamented with crenate ridges

and dot-like sclerotisations. Stoma very small, its lining is uniform with the lining of the pharynx. Pharynx distinctly subdivided into anterior corpus and posterior postcorpus; corpus 43-50 μm long, cylindrical or slightly fusiform, muscular; postcorpus 71-104 μm long, consists of anterior narrow non-muscular isthmus and pear-shaped glandular basal swelling. Pharyngeal gland nuclei indistinct, orifices of three pharyngeal glands are present at the anterior part of the pharynx. Pharyngeal lumen uniform in thickness, tubes and valve-like structures absent. Nerve ring surrounding isthmus at approximately three-fifths of pharynx length (NR = 55-61%). Secretory-excretory system present; excretory pore located along the ventral body line opposite the junction of pharynx with intestine; excretory duct very short, leading from pore to ampulla; renette cell elongate-ovoid, its body adjacent and ventral to anterior part of intestine. Tail subcylindrical. Caudal glands opening *via* three separate openings, spinneret absent.

Female. Reproductive system didelphic, amphidelphic, reflexed; ovary branches symmetrical. Anterior ovary situated to right of intestine; posterior ovary situated to left of intestine. Vulva a transverse slit immediately posterior to midbody. Vagina straight, one fifth to one third of the vulval body diameter, with thick walls and well developed sphincter muscle at its proximal part; with slight sclerotisations seen in some specimens. Intrauterine egg not seen; sperm is present in the uterus. Rectum short, 0.8-1.2 of the corresponding body diameter long.

Male. Reproductive system diorchic, both testes outstretched. Spicules paired and symmetrical, strongly arcuate, with rounded manubrium and conoid shaft. Gubernaculum plate-like with distinct caudal apophysis. One (subventral) pair of precloacal and eight (subventral) pairs of caudal setae.

Diagnosis. *D. paramastigia* sp. n. is particularly characterised by large body (1.18-1.79 mm), cuticle without longitudinal striation, no cuticular plate, wide loop-shaped amphids with sclerotised amphideal plate, long (12-18 μm) cephalic setae and posterior position of excretory pore.

Relationships. *D. paramastigia* sp. n. is most similar to *D. mastigia* in its body size (1.18-1.79 mm vs 1.54-2.48 mm in *D. mastigia*), and broad asymmetrical amphid with wide amphideal shield that is ornamented with punctuations. However,

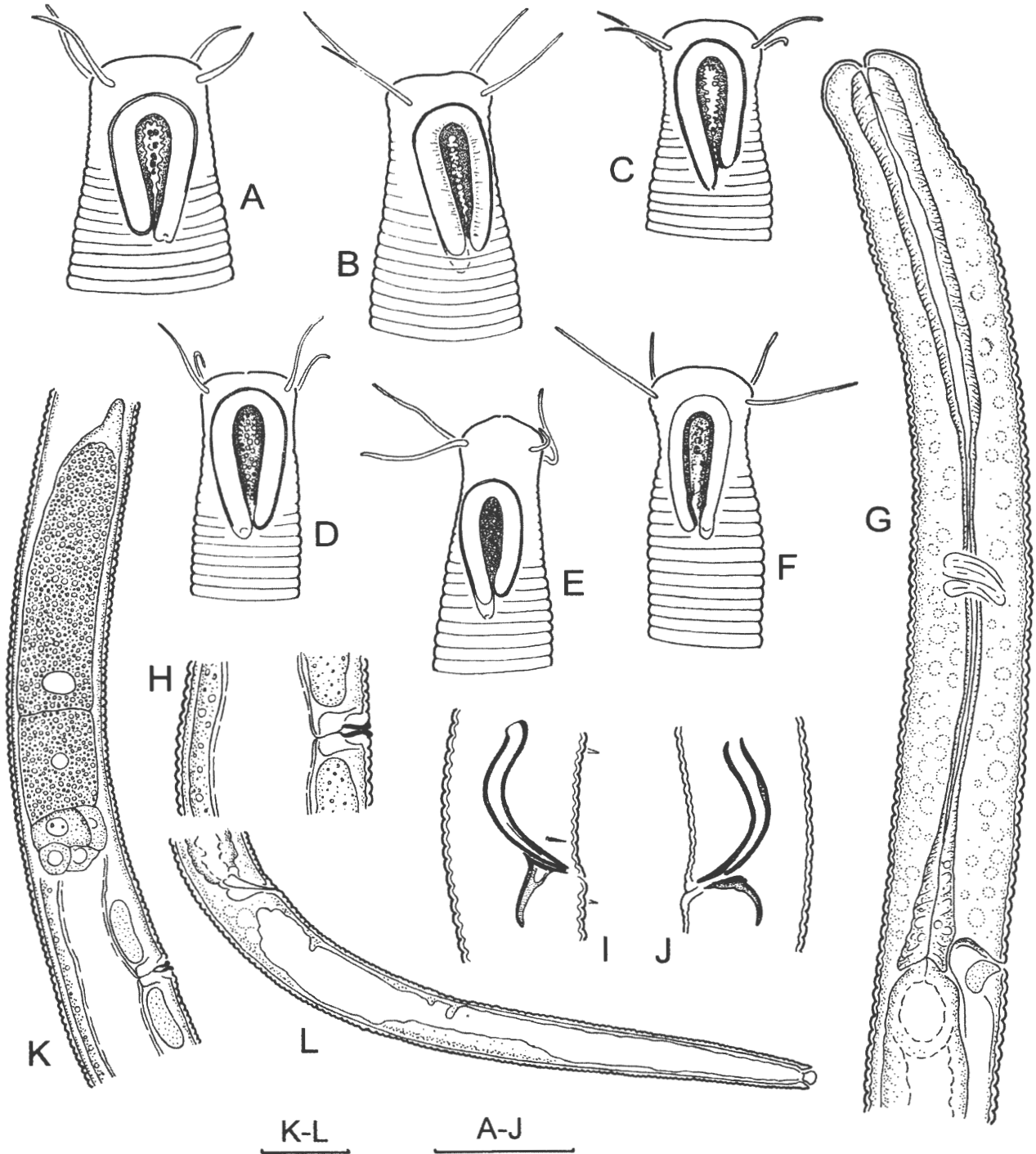


Fig. 4. *Diplopetoides paramastigia* sp. n. A-D, G-H, K-L: Female, E-F, I-J: Male. A-F: Anterior end, surface view; G: Pharyngeal region; H: Vulva; I, J: Cloacal region; K: Vulval region and anterior ovary. L: Female tail. (Scale bars: A-H = 20 μ m.).

Table 1. Morphometrics of *Diplopetoides axayacatl* sp. n., *D. paramastigia* sp. n. and *D. santaclarae*. All measurements are in μm .

	<i>D. axayacatl</i> sp. n.			<i>D. paramastigia</i> sp. n.			<i>D. santaclarae</i> sp. n.		
	HT ♀	1 ♀	1 ♂	HT ♀	2 ♀♀	1 ♂	HT ♀	4 ♀♀	2 ♂♂
L	1605	1449	1824	1975	2038; 1865	1625	1510	1175-1786	1633; 1500
Body diam.	29.0	24.0	20.5	40.0	34.0; 29.0	27.5	27.5	20.6-29.5	19.5; 22.5
Pharynx length	126	124	138	159	183; 164	155	141	109-152	150; 154
Tail length	109	110	126	155	143; 140	143	138	110-185	148; 138
Anal or cloacal body diam.	22.0	19.0	21.0	22.5	25.0; 22.5	24.0	16.0	15.0-21.0	17.5; 20.0
a	55.3	60.4	89.0	49.4	60.4; 64.9	59.1	54.9	52.2-63.4	83.7; 66.6
b	12.8	11.7	13.2	12.4	11.1; 11.4	10.5	10.7	10.3-11.8	10.9; 9.8
c	14.7	13.2	14.5	12.7	14.3; 13.3	11.4	10.9	7.1-13.6	11.0; 10.9
c'	5.0	5.8	6.0	6.9	5.7; 6.2	6.0	8.6	6.2-12.3	8.5; 6.9
V or T	56.0	51.9	?	56.2	59.4; 53.9	?	57.6	52.4-56.9	40.6; 50.1
G ₁	11.8	15.2	-	11.9	11.0; ?	-	9.8	8.3-11.9	-
G ₂	13.1	15.0	-	13.9	8.3; 13.8	-	10.8	11.8-11.9	-
Labial region diam.	12.5	14.0	13.0	17.5	15.0; 12.5	17.5	14.5	14.0-17.0	14.5; 12.5
Cephalic setae length	15.0	16.0	20.0	19.0	16.0; 19.0	17.5	13.0	12.0-18.0	16.0; 16.0
Amphid width	7.5	9.0	9.0	12.5	10.0; ?	14.0	10.0	9.5-13.0	9.5; 10.0
Dorsal amphid branch length	12.5	11.0	15.0	27.0	33.0; ?	32.0	22.5	22.5-25.5	22.0; 21.0
Ventral amphid branch length	9.0	9.0	9.0	26.0	32.0; ?	31.0	19.5	21.0-24.0	20.5; 18.0
Ant. end to excretory pore (μm)	112	112	125	147	175; 151	134	140	95-128	135; 125
Ant. end to excretory pore (%)							99	82-89	
Ant. end to excretory pore (ann.)	59	52	52	57	63; ?	55	69	51-64	73; 56
Vagina or spicule length	7.5	8.0	31-32	8.0	9.0; 7.5	29.5	7.0	5.5-9.0	28.0; 28.0
Rectum or gubernaculum length	15.0	15.0	2.5	27.5	25.0; ?	8.0	14.5	16.0-20.0	10.5; 7.0

new species differs from *D. mastigia* in longer cephalic setae (12-18 μm vs 6.5-8.5 μm in *D. mastigia*) and presence of gubernaculum apophysis (vs absent in *D. mastigia*). *D. paramastigia* sp. n. shows similarities to *Diplopetula botula* in body size (1.18-1.79 mm vs 1.66 in *D. botula*) and long cephalic setae (12-19 μm vs 17 μm in *D. botula*), but is clearly distinguished by the presence of punctations on the amphideal shield (vs absent in *D. botula*) and position of excretory pore (EP = 82-99% vs EP ca. 60% in *D. botula*). Compared to both other new species described above, *D. paramastigia* can be distinguished by its smooth cuticle (versus striated) and longer rectum (25-28 μm vs 20 μm or less).

Type locality. Mexico, Gulf of California, Punta Estrella (including San Felipe), samples taken in 2002 (holotype, one female and one male).

Other locality. Mexico, Gulf of California, Santa Clara (including El Tornillal), samples taken in 2006, 2007 and 2008 (three females and one male paratypes)

Type material. Holotype female, four females and one male deposited in the University of California Riverside Nematode Collection, CA, USA, one male paratype deposited in CICESE, Ensenada BC, Mexico.

Ethymology. The specific epithet refers to the close resemblance of the new species to *D. mastigia*.

NOTES ON THE GENUS *DIPLOPETULA* GERLACH, 1950

D. breviceps, the type species of the genus *Diplopetula*, is known on the basis of females and males, but no data are available about the structure of the ovaries for this species, while "the lumen of the pharynx widens anteriorly only very slightly" (Gerlach, 1950). Partly as a result of this incomplete description of the type species, the genus *Diplopetula* ended up including over twenty species by now, which are "quite different from each other so that it is rather difficult to establish a concise generic diagnosis" (Vincx & Gourbault, 1992).

Table 2. Selected diagnostic characters of species of the genus *Diplopetoides* based on literature data supplemented with recent observations.

	Body length (mm)	Cuticle striation	Amphid shape	Inter-amphidial shield	Cuticular plate	CSL (µm)	EP (%)	Ovaries	SPIC (µm)	Special features
<i>Diplopetoides</i>										
<i>D. ornatus</i>	0.43-0.49	present	narrow, symmetrical	absent	present	4	~55%	reflexed	22	
<i>D. linkei</i>	0.48-0.50	present	narrow, symmetrical	absent	present	2-3	?	reflexed	29	
<i>D. anatolii</i>	0.93-1.02	absent	narrow, asymmetrical	absent	absent	2	?	reflexed	40, on chord	
<i>D. mastigia</i>	1.54-2.48	absent	wide, asymmetrical	punctated	absent	6.5-8.5	?	reflexed	25-37	
<i>D. pulcher</i>	1.07-1.15	present	wide, asymmetrical	smooth	absent	16	49-56%	reflexed	25	
<i>D. sundensis</i>	1.12	absent	wide, asymmetrical	smooth	absent	5	~90%	NA	25	two supplements
<i>D. nudus</i>	0.94-1.35	absent	narrow, asymmetrical	absent	absent	5	~80%	?	29	
<i>D. axayacatli</i> sp. n.	1.45-1.82	present	wide, asymmetrical	crenate to punctate	present	15-20	89-91%	reflexed	31-32	amphid hook-shaped
<i>D. santacлару</i> e sp. n.	1.62-2.04	present	narrow, symmetrical	crenate	absent	17.5-19	92-96%	reflexed	29.5	gubernaculum apophysis
<i>D. paramastigia</i> sp. n.	1.18-1.79	absent	narrow, symmetrical	punctate to crenate	absent	12-18	82-99%	reflexed	28	gubernaculum apophysis
<i>Diplopeltula incertae sedis</i>										
<i>D. botula</i>	1.66	absent	wide, symmetrical	smooth	absent	17	~60%	?	NA	
<i>D. striata</i>	1.74	present	wide, asymmetrical	smooth	absent	12-15	~90%	?	17	spicula shape
<i>D. lucanica</i>	1.22-1.50	absent	narrow, asymmetrical	crenate	absent	11-16	?	?	28	

At least seven species are known from males only: *D. belgica* Vincx & Gourbault, 1992, *D. cylindricauda* (Allgén, 1932) Gerlach, 1950, *D. curta* Vincx & Gourbault, 1992, *D. laminata* Vitiello, 1972, *D. minuta* Vitiello, 1972, *D. pumila* Vincx & Gourbault, 1992 and *D. striolata* Vincx & Gourbault, 1992. Thus, no data about the female gonad morphology are available for these species. As for the other species of *Diplopeltula*, only descriptions of *D. incisa* and *D. indica* Gerlach, 1962 clearly state the morphology of the ovaries, which are outstretched.

In the discussion below we will analyze the taxonomic status of each species. Those species for which the systematic position (affinities to either *Diplopeltula* or *Diplopeltooides*) cannot be established definitively, will be discussed and compared with our new species further below.

1) In the original description of *D. curta* the authors stated that this species is unique within the genus *Diplopeltula* by its posterior position of the cephalic setae and by its amphideal fovea, which is ventrally wound and loop-shaped with oval contour (Vincx & Gourbault, 1992; Fig. 4). However, it is similar to the genus *Pararaeolaimus* Timm, 1961 in these respects, particularly to *P. nudus* (Gerlach, 1956) Timm, 1961 (type) and *P. rumohri* Jensen, 1991. All these species are morphologically very similar and are characterised by the short barrel-shaped stoma, cylindrical and uniformly muscular pharynx, four cephalic setae located posterior to the base of stoma, large amphids in the shape of wide oval loop, paired and outstretched ovaries, and other characters (Gerlach, 1951; Jensen, 1991; Lorenzen, 1973; Tchesunov & Miljutina, 2008). We therefore propose a new combination: *Pararaeolaimus curtus* (Vincx & Gourbault, 1992) **comb. n.** The systematic affinities of *P. megalamphidius* Timm, 1961 cannot be evaluated with confidence, since the original description is based on single immature female (Timm, 1961).

2) *Incisa* species group. The following species form a closely related group: *Diplopeltula incisa*, *Diplopeltula bathmanni* (Jensen, 1991) **comb. n.** and *Diplopeltula onusta*. They share a dorsally located oral opening, a relatively large buccal cavity, asymmetrically located cephalic setae and an amphid located on the cuticular plate (Gerlach, 1950; Jensen, 1991; Sergeeva, 1974; Southern, 1914; Vitiello, 1972; Voronov, 1982; Wieser, 1956). As stated above, *D. onusta* was synonymized with *D. incisa* by Voronov (1982). See Voronov (1982) for other synonyms of *D. incisa*. These species differ from all members of the genus

Diplopeltooides particularly in the arrangement of cephalic setae (asymmetrical vs symmetrical in *Diplopeltooides*), position of oral opening (dorsal vs apical in *Diplopeltooides*), structure of stoma (wide barrel-shaped vs absent in *Diplopeltooides*), pharynx (cylindrical vs with narrow isthmus in *Diplopeltooides*), and female reproductive system (ovaries outstretched vs reflexed in *Diplopeltooides*). They also differ from the type species of the genus *Diplopeltis* Cobb in Stiles & Hassal, 1905, *D. cirratus* (Eberth, 1863) Cobb, 1891, particularly in the arrangement of cephalic setae (asymmetrical vs symmetrical in *Diplopeltis*), position of oral opening (dorsal vs apical in *Diplopeltis*), structure of stoma (wide barrel-shaped vs absent in *Diplopeltooides*), absence of ocelli (vs present in *Diplopeltis*), caudal glands opening separately (vs via a common spinneret in *Diplopeltis*). Different species and specimens were described as having or not having subcephalic setae at level of amphid.

3) *Laminata* species group. Another three species form a closely related group: *Diplopeltula laminata* Vitiello, 1972, *D. cassidaignensis* Vitiello, 1972 and *D. cylindricauda*. These share presence of subcephalic setae located in four sublateral rows at level of amphid (Allgen, 1932; Vitiello, 1972). Therefore, they differ in this respect from the genus *Diplopeltooides* and most species of *Diplopeltula* except for the *incisa*-group. At the same time they resemble *Diplopeltis cirratus* in the presence of cervical setae and caudal glands opening through the common spinneret, differing from it in the number of cervical setae, absence of cuticular plate and ocelli.

4) *Diplopeltula intermedia* Gerlach, 1954 and *D. indica* are very similar to each other in most morphological and morphometric characters except for the presence of a cuticular plate (present in *D. indica* "situated on a cuticular plate" vs absent in *D. intermedia* "since lateral organs are not situated on cuticular plates, I assign the species to the genus *Diplopeltula*." (Gerlach, 1954, 1962). They both differ from the genus *Diplopeltooides* in the presence of barrel-shaped stoma (vs absent in *Diplopeltooides*), cylindrical pharynx (vs with narrow isthmus in *Diplopeltooides*) and outstretched ovaries (vs reflexed in *Diplopeltooides*).

5) Three other species currently assigned to *Diplopeltula* possess a dorsally shifted oral opening: *D. belgica*, *D. ostrita* Boucher & Helléouët, 1977 and *D. striolata*. *D. ostrita* is known from females and males, *D. belgica* and *D. striolata* were described on the basis of one male each. No data about the structure of ovaries are available for any

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of these three species. *D. ostrita* is unique within the genus by its very long tail and shows similarities to the genus *Campylaimus* Cobb, 1920 (see Boucher & Helléouët, 1977). *D. belgica* differs from every other species of *Diplopettula* and *Diplopettoidea* in the shape of amphid with the *porus amhidualis* located at the anterior end of the loop, the U-shaped loop directed posteriorly and excretory pore opening anteriorly to cephalic setae (Vincx & Gourbault, 1992). *D. striolata* appears to differ from every other species of *Diplopettula* and *Diplopettoidea* in the position of the excretory pore posterior to the corpus-isthmus junction (Vincx & Gourbault, 1992). All three species are distinctly different from the genus *Diplopettoidea* in its current sense.

6) *D. bulbosa* Vitiello, 1972 and *D. nellyae* Vincx & Gourbault, 1992 were both described from males and females, but without information about the structure of the ovaries. "oth species are characterised by the uniformly cylindrical pharynx with well developed muscular basal bulb, thus being sufficiently different from the genus *Diplopettoidea* (Vitiello, 1972; Vincx & Gourbault, 1992). Four other species were clearly described as having cylindrical pharynx, sometimes with basal swelling or pear shaped bulb: *D. asetosa* Juario, 1974, *D. longiceps* Gerlach, 1950, *D. minuta* Vitiello, 1972 and *D. pumila* Vincx & Gourbault, 1992. In this respect they all differ from the genus *Diplopettoidea*. Furthermore *D. minuta* is the only species within this group with clavate tail.

Descriptions of the following lack information about the pharynx and female reproductive system morphology: *D. botula* (Wieser, 1959), *D. lucanica* Boucher & Helléouët, 1977 and *D. striata* Gerlach, 1956. The description of *D. botula* (originally described in the genus *Araeolaimoides* de Man, 1893) was based on a single female. All three are considered *species incertae sedis* and were compared with new species of *Diplopettoidea* above (see Table 2).

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O. Holovachov, I. Tandingan De Ley, M. Mundo-Ocampo, R. Gingold, P. De Ley. Нематоды Калифорнийского залива. Часть 3. Три новых вида рода *Diploeltooides* Gerlach, 1962 (Nematoda: Diploeltooididae) и обзор родов *Diploelptis* Gerlach, 1962 и *Diploelptula* Gerlach, 1950.

Резюме. По данным световой микроскопии описано три новых вида: *Diploeltooides axayacatl* sp. n., *D. santaclarae* sp. n. и *D. paramastigia* sp. n.. Все три вида по морфометрическим данным близки к *D. mastigia*, хотя *D. axayacatl* sp. n. отличается крюковидными амфидами. Два последних вида отличаются от *D. mastigia* наличием апофиза на рульке, а друг от друга строением кутикулы (исчерченная и гладкая, соответственно) и наличием соответственно пунктуации или гребней на интерамфидиальном поле. Обсуждается статус и состав рода *Diploeltooides* и проводится его сравнение с *Diploelptis* и *Diploelptula*. Несколько видов переведены в другие роды. *Diploelptula curta* перемещен в род *Pararaeolaimus*; *Diploelptis bathmanni* переведен в *Diploelptula*, *Diploelptula pulcher* и *D. sundensis* – в род *Diploeltooides*. Три вида рода *Diploelptula* рассматриваются как *species incertae sedis*: *D. botula*, *D. striata* и *D. lucanica*.
