DESCRIPTION OF *DIPLOLAIMELLA DIEVENGATENSIS* SP. N. (NEMATODA: MONHYSTERIDAE)

BY

L. J. JACOBS, M. C. VAN DE VELDE, E. GERAERT and G. VRANKEN

Laboratorium voor Morfologie en Systematiek der Dieren, Rijksuniversiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium

Diplolaimella dievengatensis sp. n. was sampled from the Dievengat, a thalassic water pond situated near Knokke in North Western Belgium. The different juvenile and adult stages are described in detail. The male of *D. dievengatensis* is characterised by the length of the spicules $(>25 \,\mu\text{m})$, the prominence of the gubernacular apophyses and the presence and position of five pairs of equal and prominent post-cloacal genital papillae. The female is characterised by the sclerotizations of the vaginal cuticle and the absence of a postvulval uterine sac.

Keywords: taxonomy, morphology, brackish water, nematodes.

Diplolaimella dievengatensis sp. n., a bacterivorous monhysterid, was misidentified in previous papers as Monhystera microphthalma de Man, 1880 (see below). The species is easily maintainable in laboratory cultures (Vranken & Heip, 1986b).

In this paper, specimens sampled from cultured populations are described in detail, including the different juvenile stages. It was originally drafted by L. J. Jacobs and G. Vranken, but both were unable to continue. This caused much delay in publication of the article and, as a consequence several authors used this species name as a *nomen nudum* (listed below).

MATERIAL AND METHODS

Diplolaimella dievengatensis sp. n. was collected from the "Dievengat", a shallow polyhaline brackish-water pond in North Western Belgium. Sediment samples were immediately treated in the laboratory. The organisms and organic debris were separated from the sediment with Barnett's method (Barnett, 1968), using water of the Dievengat, and collected on a sieve with a mesh width of 38 μ m. Vented petri-dishes with a diameter of 14 cm were filled with 0.8% bacto-agar (DIFCO) made up with Dievengat-water of ca. 20 g per I salinity. After cooling, four excavations were made in the agar-medium with a spoon, and elutriated material was placed in three of the four excavations. The remainder was filled with pure sediment. After an incubation time of four weeks, benthic organisms, including nematodes, started to colonize the agar. These detritus spot-agar dishes can be maintained for a couple of

months. Adults of D. dievengatensis sp. n. were sampled from such cultures.

Monospecific cultures of *D. dievengatensis* sp. n. were started by transferring gravid females to enriched bacto-agar medium. The medium used for experiments and stock-cultures is a 0.8 sterile bacto-agar (DIFCO) to which 1% medium of Vlasblom (cf. Vranken *et al.*, 1984a) and 0.5-1.0 Na₂ SiO₃.9H₂O (0.053 M stock-solution) was added. The salinity of cultures was maintained at about 20 g per l by adding distilled water when necessary. An unidentified bacterial mixture originating from the Dievengat, grown separately in Erlenmeyer flasks filled with sterilized Dievengat-water and enriched with Vlasblom medium, was added in abundance.

The nematodes were fixed by adding 4% formaldehyde at 70°C to embryodishes containing the living worms in Dievengat-water. These specimens were then processed for mounting in anhydrous glycerine and studied with a Leitz Dialux 20 EB phase contrast interference light microscope. Series of cross sections of glycerine-gelatine embedded adults were made manually. For the S.E.M. studies formalin fixed specimens were embedded in Spurr's resin (Spurr, 1969; Clark & Stone, 1975), coated with I.S.I. PS-2 and examined with a JEOL JSM-840.

DESCRIPTION

Diplolaimella dievengatensis sp. n. (Figs. 1-11; Table I)

syn. Monhystera microphthalma nec de Man, 1880

Geraert et al., 1981 p. 149, 153-154 Fig. 2A, D

Bogaert et al., 1984 p. 21-29

Samoiloff and Bogaert, 1984 p. 415

Vranken et al., 1984a p. 159-180 Tabl. II-V Fig. 1 Vranken et al., 1984b p. 285-286

Heip et al., 1985a p. 15, 32-36 Tab. 2-5 Fig. 12

Heip et al., 1985b p. 403, 451, 457 Tab. VIII,

XI-XIII Fig. 11

Vranken et al., 1985 p. 49-56 Tab. 1-3 Fig. 2-3 syn. Diplolaimella sp. 1

^{*} Vranken & Heip, 1985 p. 167-269, 271, 276-277 Tab. 1-2 Fig. 2

Vranken & Heip, 1986a

Vranken et al., 1986 Tab. I

Diplolaimella dievengatensis as nomen nudum

Van de Velde & Coomans, 1988, 1989, 1990

Van de Velde & Coomans (in press)

Vranken, 1987

Vranken & Heip, 1986b p. 431, 436 Tab. 1-3, 5

TABLE I

Diplolaimella dievengatensis sp. n. Measurements and diagnostic morphometric features

**	225	5000-0 000-0	57.7 58.9 76.7 1.2			
o min-max	5 0.61-0.51 0.42-0.21 0.42-0.721 0.271-0.721	57-67 72 0-13 A 3 2-13 A 9 5-10 1 17 0-20 0 18 0-19 1	72 0.455.0 445 0.550.0 788 0.922 0 543 0.754 0 543 0.754 0	42.0-72.0 7.2-6.0 35.6-80.0 43.0-57.0	28-31 1 8-24 1 8-24	1,4-1,5
DEPU	1030 4 21 8 225 2 2 23 2	9-8449 920698	77 8 560.0 838 0 655 1 27 0	4.7 7.7 5.7.8 5.7.8	200	12.0
*	a surg	2000000000	19892811			
ç min mın	10 948-1259 22.0-29.0 121.0-140.0 263.0-384.0	6.27.1 10.0-12.9 2.6-3.4 9.7-10.8 17.0-22.1 9.5-14.3	64 0-19.0 248 0-390.0 443 0-591.0 245.0-114 0 245.0-114 0 245.0-13 0	0.0.47 0 7.6-9.1 3.3-3 1.2 0-57-0 6.0-44-0 5.0-31.0	2.9-9.7 1.5-2.0 9.9-13.0	15.028 0.5-028
nean	1126.6 26.9 726.9 736.7	661 100 100 100 100 100 100 100 100 100	1 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	0 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9	1 8 9 3 3	990 1990 1991
	888 899 899 899 899 899 899 899 899 899	0.0 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1	21 - 1 86 1			
J+	5 776-948 16.0-23.0 120.0-135.0 120.0-230.0	4,4-5.7 9.0-12.6 1.9-2.9 7,0-9.1 12:0-15.0	57 0-75 0 145 0-396 0 438 0-640 0 (a = 1) (a = 2)	35 0-19.0 6 0-0,9 3,5-1,3 3,5-1,3 3,5-5,6,0 56:0-79.0 56:0-79.0	51-3,6 18-2,5 9,5-19.5	12,4-13.6 0,5-1 1
mean	809 d 22 2 23 5 24 5 21 4 2	202864 2028 2027 2027	20.8 278.6 590.4 125.0	40 6 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	108 108	14.8 0.8
-	23.9 25.0 25.0 25.0	0.1 0.8 0.8 0.8 0.8	2.28 2.28 2.28 2.58 2.58 2.58 2.58 2.58			
J3. min max-	5 510-579 15.0-27.0 07.0-112.0	1.8-5.7 11.0-13.6 2.0.7.5 7.8-9.2 13.0-15.0	59.0-68.0 50.0-88.0 198.0-417.0 299.0-183.0 71.0-17.0	19.0-37 0 7-5.1 3 7-4.0 8.0-79.0 9.0-17 0	2.3.4 7.9-9	0.3-1,0
mean	533 6 108 2 111.0	47846 4 8 4787€68	2000 2010 2010 2010 2010 2010 2010 2010	816 84 85 85 85 85 85 85 85 85 85 85 85 85 85	1472	0 9 0 8
4	86.7 1.8 8.7 22.2	886 982 5	000			
J1 Inar max	5 263 145 9.0-133 0 80.0-102.0	1,95,6 1,52,4 1,52,4 5,48,6 5,48,6 5,48,6 5,48,6 2,0,0,0 2,7,9,6	41,0-63,0 10,0-28,0 153,0-286,0 62,0-120,0 65,0-26,0	26 0 34 0 3 2 4 0 3 8 4 1 5 0 62 n 7 0 82 0 4 0 6 0	5246 5425 6425	111-02 111-02 111-02 111-02 111-02
nteatr.	346.0 11.4 90.6 98.0	*5-~5%~ 2.000.0000	266 2666 2666 2666	30.2 3.8 5.2 62.8 62.8	1987 1987	100 - T
en.	18.0 9.0 5.4	20000000	1199911			
11 nun-nuax	5 286-335 12.0-12.0 81.0-92.0 73.0-85.0	3.84.5 11 5-13 7 1.1-1 7 7.0-7 7 8.1-9.5 5.4-6.8	70-15.0 70-12.0 72.0-169.0 72.0-80.0 59.0-75.0	25.0.27.0 3.8+1.8 50.058.0 49.051.0 2.0+0	4 0 5 2 2.6 3 2 6.5 7 2	8 2-9 5 8 2-9 5 0 7 0 8 0 9-1 1
Incan	306.0 85.2 77.8	6817400 64779 64779	1 66 1 66 1	200800 24080 24080 200800 200800 200800 200800 200800 200800 2008000	9 6 6 6 6 6 6 6 6	880 10 10 10 10
	L m.b.« t.L	1.4 1.4 1.6 1.6 1.6 1.6 1.6 1.1 1.1 1.1 1.1 1.1	π.r 8 V or V V add, or V ad, 10 Vd. or P ad, 10 Vd. or Ph V, 1 ph dd.	> 215 112>0	f.b ~//.d h.1./h d ph.1./h.1.	val /a.b.wc' t.l./a.b.wc' r.l./a.b.w. sp.l./a.b.w. t.l./a.a.l.

DIPLOLAIMELLA DIEVENGATENSIS SP. Ν

pharynx-cardia purction and vulva (µm); ph.v'l.: distance berween pharynx-cardia junction and posterna margun of genical primordium (µm); r l.: rectum length (µm); xp.l. #picule Ringth measured along the arc (µm); t.l. Inil length (µm); v: distance berween anartivo body end and vulvn; V' instance between anartivo francing of genical Abbreviations to Table I. a.b.w.: anal body width (µm); c.b.w.: cordial body width (µm); I.b w.: avrage of the body width µt he scatte of bulh amphideal foveae (µm), I.d.: over ovea aperture diameter measured parallel to central hody axis (µm)); h.d.: head diameter = body with a cephalic selae measured perpendicular to central body axis (µm); h.d.: head cueth = distance between anlerior body end and anterior margin of both amphile-floyree, meatured along central both and (mm); [1] [4; juy-mle stage, 1-4; m.b.w. m. stmal both diameter (µm); n: number of specimens measured; pli.cl.]; distance between plustyne cardia junction and cloacal orifice (µm); pl.A.1; pharyna longth (µm), ph.V.1; distance between primordium (µm), V: distincte between anterior buildy end and vulva as percentage af body length (%); V*: distance between anterior body cut and posterior margin of genical poincedium as percentage of body length (% , , a.l.: divince between vulva and mu (am); v, a.l., distance between posterior marrin at primordium and new (am). 4

Females. Body about 1 mm and slender; body diameter almost equal between nerve ring and anus, tapering anteriorly from nerve ring to 50% at amphideal fovea and 30% at cephalic setae; head rounded to slightly truncated; tail long and filiform. Body cuticle striated (Fig. 11) and relatively thick (at fovea: 0.8 µm; between cardia and anus: 1 µm; at spinneret: 0.5 µm). Three layers are distinguishable in optical section: (1) external (cortical) layer: finely striated, annuli 0.5-0.9 µm; (2) median layer: thin and (3) internal (basal) layer. Body cuticle bifurcates anteriad of cephalic setae: internal part of cuticle constitutes the endocupola (Belogurov, 1985) and external part of cuticle forms the lip cuticle. Intracuticular cephalic cavity normally optically empty, but sometimes extensions of anterior sensillae are visible. Somatic setae fine, 2 µm long, ventro- and dorso-sublaterally distributed and alternating in neck region; foremost somatic seta situated at base of fusus amphidialis. Epiderm thin between chords; the lateral chords are 25-30% of corresponding body width. Optically empty ducts are situated in the chords (arrows: Figs 4N, 5D, 5G). Somatic muscles meromyarian and platymyarian, two muscle cells in each sector in neck and tail region, and 2-4 muscle cells in each sector in rest of body. The pseudocoelome is optically absent in neck region, hyaline, turgescent, ovoid cells are situated between pharynx and body wall. Six large, turgescent cells with large nucleus are present around cardia, at the base of neck. In live specimens, the cells are easily visible and dark; in preserved specimens (4% formaldehyde), they are hyaline, not granulated.

Amphid cryptospiral, ventrad wound (Figs 3E, 4 M-P). Foveal aperture circular to slightly oval with a diameter of 2.6-4 μ m (i.e. one third of corresponding body diameter). The anterior margin of fovea aperture is situated at annuli 20-30 (about 1.5-2.0 body diameters from anterior body end). Maximal diameter of fovea larger than fovea aperture, fovea basin unispiral (Fig. 11d). Canalis amphidialis surrounded by large, clearly visible cell (*cf.* supporting or socket cell: Coomans, 1979). Fusus length ca 1.0-1.5 × foveal diameter. One pair of ocelli, dorso-lateral of pharynx, located at 37-50 μ m from anterior body end (n = 8) i.e. at 30-40% of pharynx length or at 5.6-8.1 × head diameter from anterior body end. In live material, ocelli red-violet and well visible at low magnification; in preserved specimens, ocellus 'lens' very refractive and the colour of the pigment pouch has disappeared.

Lip region (lateral view): lips not set off, small protrusion at oral orifice sometimes visible (Figs 2D,H,I,J, 3A,B). Lip region (*en face* view): lips amalgamated; maximal oral orifice 1.5 μ m diameter, variable in shape. Six inner labial sensillae papilloid, situated median on lips at the margin of hexaradial, disk-like elevation around oral opening (Fig. 11C). Six outer labial sensillae setiform, ca 0.8 μ m long and four cephalic setae, ca 0.6 μ m long, all arranged in one circle.

Stoma composed of two bipartite buccal chambers. First buccal chamber well sclerotized, ca $3.5 \ \mu m \log n$, maximal diameter $2.0-2.5 \ \mu m$. Anterior part

Fi

DIPLOLAIMELLA DIEVENGATENSIS SP. N.



Fig. 1. D. dievengatensis sp. n. Lateral view of the tail: A - J1; B - J2; C - J3; D - J4 φ; E - φ; F - φ; G - J4 σ; H - σ; I - late J2 (cf. Figs 2E and 6C); J - σ; K - tail tip φ.

JACOBS, VAN DE VELDE, GERAERT & VRANKEN



Fig. 2. D. dievengatensis sp. n. Anterior region A, D-K: internal structures; B-C: surface view; A - J1; B - J1; C - J2; D - J3; E - late J2; G - early J1; H - J1; I - late J1; J - J3; K - J3.

j

DIPLOLAIMELLA DIEVENGATENSIS SP. N.



Fig. 3. D. dievengatensis sp. n. A-B: lateral view of anterior region J4; A - surface view; B - internal structures; C - lateral view of anal region of Q; D - ventral view of anal region of J4; E surface view, lateral view of anterior region of Q (cf. Fig. 4Q).

of first buccal chamber (cheilostome) sloping anteriad; posteriad twice as wide as oral orifice. Posterior part of first buccal chamber funnel shaped, lumen from triangular to rounded. Second buccal chamber anteriorly strongly and posteriorly less strongly sclerotized, bullet-shaped, ca 2.0 μ m long and maximal 2.5 μ m wide; lumen triradial. JACOBS, VAN DE VELDE, GERAERT & VRANKEN



Fig. 4. D. dievengatensis sp. n. A - lateral view of anterior region of Q (cf. Fig. 3E); B - lateral view of anterior region of Q; C-P - optical cross sections through anterior region.

Pharynx cylindrical, muscular; pharyngeal bulbus absent; anterior- and posteriormost pharyngeal regions more hyaline than in remainder of pharynx. Pharyngeal musculature starts anteriorly immediately after the cheilostome. Optical cross sections of pharynx: three pairs of prominent adradial muscles; three pairs of perradial fibrillar strings. Three pharyngeal gland cells present: one dorsal and a pair of ventrosublateral ones. Nucleus of dorsal gland cell, at ca 1.5 times the cardial body width anteriad to pharynx-cardia junction (Figs 10H,K: dn). Dorsal gland contains larger granules than ventrosublateral ones; ampulla present at anteriormost part of gland duct at ca 2 μ m posteriad of second buccal chamber. Nuclei of ventrosublateral glands situated at ca one cardial body width in front of the pharynx-cardia junction (Figs 10H: S₂N₁,





Fig. 5. D. dievengatensis sp. n. A - Q; B - σ ; C-G - optical cross section through pharynx and cardia, arrows indicate epidermal tubes; H - lateral view of posterior part of pharynx and cardia of Q.

10J: S_2N_1 , S_2N_2) both nuclei at the same level or left nucleus somewhat posteriad of right one. Another pair of smaller, but prominent nuclei are located just behind the nerve ring; both nuclei at the same level or the left one somewhat posteriad of right one (Figs 10I: S_1N_1 , 10K: S_1N_1 , S_1N_1); these were first interpreted as gland nuclei.

Cardia rhomboid, 11 μ m long, lumen lined with cuticle. Three zones distinguishable (Fig. 5G, H): (1) apical pharyngo-cardial transition zone, situated at conoid invagination of cardia in pharynx, surrounded by a fibrillar ring (sphincter ?, Fig. 10I: cc), lumen triradial; (2) median zone consisting of two circles of cardial cells (Fig. 10G: ec, lc), cardial cells with granules, fibres and many nuclei (2 × 4 or 2 × 6), cell membranes indistinct (syncytium ?), lumen shape changing posteriad from triradial to bilateral due to shortening/disappearance of ventral branch of lumen and widening of dorsal sector; (3) intestinal zone situated at invagination of cardia in intestine, consisting of dense differentiation of central set of cardial cells.

Nerve ring situated at 64-78% of pharyngeal length, often difficult to distinguish.

Ventral gland, ventral to subventral on the right side of intestine, caudal of progaster. Ampulia of ventral gland mostly prominent, situated at level of ocelli; with apically, short sclerotised excretion duct and outlet located in center of small and shallow basin (Fig. 10E).

Progaster present, anteriormost intestinal cells smaller, less granulated than other intestinal cells. Intestine, a straight tube, lumen small, intestinal cells arranged per two in circumference, filled with large granules, zone of microvilli 3-5 μ m wide. Rectum slit-shaped in optical cross section, lined with cuticle, length 0.5-0.8 × anal body diameter, dorso-ventrally flattened, caudalmost intestinal cells with anterio-axial directed flap-shaped differentiations forming intestino-rectal valve, sphincter situated at intestino-rectal junction, dorso-caudal rectum dilator present, associated glands not found. Anus 2.0-2.5 μ m wide, transverse slit shaped.

Tail tapering gradually (Fig. 1), first third more conoid, last two thirds filiform. Three caudal glands open through common duct, spinneret with relatively large terminal cavity (Fig. 1K).

Reproductive system monodelphic, prodelphic, associated glands not found. Ovary outstretched, right of intestine, relatively long: more than twice the oviduct-uterus length. Three zones distinguishable: (1) germinative zone with oocytes arranged in three rows (Fig. $6F: 3 \times 9$ cells), cytoplasm with very thin granules, proportion karyoplasm to cytoplasm large; (2) growth zone with cells arranged in two rows, volume karyoplasm and cytoplasm increases proportionally; (3) ripening zone longer than previous zones, single row of oocytes, volume karyoplasm constant, important increase of vitelline volume. At least five nuclei present in ovary epithelium: one top cell, two lateral and two caudal ones. Large, associated coelomocytes present along ovary at variable loci.

DIPLOLAIMELLA DIEVENGATENSIS SP. N.



Fig. 6. D. dievengatensis sp. n. Reproductive system A - genital primordium of J1; B - idem of early J2; C - idem of late J2; D - idem of J3; E - idem of J4 Q; F - reproductive system of mature Q; G - subventral view of vulva.



Fig. 7. D. dievengatensis sp. n. A - lateral view of reproductive system of mature Q, uterus with large number of spermatozoa; B - idem, subventral view, uterus with egg; C - idem, ventral view, spermatheca with large number of spermatozoa; a.d.vu.: anterior dilator of vulva, c.uv.: constrictor at uterus-vagina junction, c.va.: constrictor of vagina, c.vu.: constrictor of vulva, d.va.: dilator of vagina, p.d.vu.: posterior dilator of vulva, s.m.: somatic transverse muscle, s.va.: suspensor of vagina.

Ovary sometimes slightly protruding into oviduct. Oviduct composed of at least three, probably four rows of four cells; oviduct cells swollen ovoid, cytoplasm with very fine granules and often hyaline in preserved specimens; lumen small, without spermatozoa. Uterus variable in length, partly ventral to lateral of oviduct (Fig. 7A); uterine cells coarsely granulated, with mostly one thin-shelled (0.5 μ m thick) egg at a time. Uterus often filled with up to 100 spermatozoa. Postvulval sac absent; vagina straight, short, tube-shaped, lined with cuticle, vagina-uterus junction dorso-caudal of uterus; vagina bipartite: proximal part, from uterus to vagina suspensor, with small lumen (Figs 7A,B: s.va.), vaginal dilator present at dorso-caudal side (Figs 7A,B: d.va.); distal part with slit-shaped, dorso-ventrally flattened lumen, from vaginal suspensor to vulva, surrounded by vaginal sphincter (Fig. 7B: c.va.), vaginal cuticle well developed, with anterio-ventral and posterio-dorsal sclerotizations. Caudal of vagina, at least three associated cells present of which one has a large refractive inclusion. Vulval sphincter and V-shaped vulval dilators situated just below epiderm (Figs 6G, 7C: c.vu., a.d.vu., p.d.vu.); vulva transverse crescentshaped, lateral tips of vulva directed caudally, lateral sides of anterior vulva lips flap-like, covering vulva partly.

Males. Resembling females except for the following features:

Body more slender; fovea aperture larger and more posteriad; tail shorter and first quarter conoid. One precloacal ventro-median supplement, with centrally situated papilla (Figs 11f, 11g). Five pairs of postcloacal papillae, apical with setoid structure, distance between first four pairs of papillae almost equal, fourth and fifth pair form pseudo-bursa.

Reproductive system monorchic, testis outstretched and situated right of intestine; top of testis at 2-3 body diameters from ventral gland; testis tripartite: (1) germinative zone with spermatogonia arranged in three rows, (2) growth zone with spermatocytes arranged in two rows, (3) seminal vesicle filled with 30-40 granular and some agranular cells, vesicle wall consists of thin epithelium. Spermatozoa globular, nuclear material dark, cytoplasm radially granulated (cf. Fig. 7A, C). Vas deferens relatively long, with very fine granules, lumen very small. Two, clearly visible, ejaculatory glands present on right side of intestine and vas deferens (Fig. 8A), which enter ventrally into cloaca.

Spicules equal in length; shaft bent and sclerotized; velum weakly developed. Gubernaculum 7-9 μ m long (along arc at dorso-caudal side), tube-like and with two dorso-lateral and one ventral part; lateral apophysal plates, 4-5 μ m long; 10 μ m long (Fig. 9A). Gubernaculum muscles (Fig. 9E-F) consist of a rotator (ro.g) protractors (av.p.g.; p.g.a) and retractors (c.r.g., l.r.g pv.p.g). Spicule muscles (Fig. 9E-F) consist of protractors (d.p.sp., ld.p.sp, v.p.sp) and retractor (r.sp).

Juveniles. General morphology of juveniles similar to adults (Figs 1, 2, 3). During ontogeny, body becomes relatively thinner (cf. a values in Table I);



relative pharynx length decreases (cf. *b* values in Table I); tail length almost isometric with body length except for J4 \heartsuit and \heartsuit , both with relatively shorter tails (cf. c values in Table I, J4 \heartsuit = 4.2). Distance between anterior body end and anterior margin of fovea amphidialis almost constant during ontogeny, but diminishes gradually relative to head diameter (cf. h.i./h.d. values in Table I). Diameter of fovea aperture increases relative to corresponding body diameter (cf. f.b.w./f.d. values in Table I).

Genital primordium of J1 ca 10 μ m long, consisting of two large germinative cells and two flattened somatic cells (Fig. 6A). J2 primordium ca 10-28 μ m long which has in early J2 four germinative cells and two flattened somatic cells (Fig. 6B), and in late J2 the somatic cells proliferate: 2×3 undifferentiated somatic cells and posteriorly two vaginal cells (Fig. 6C). J3 primordium ca 50-80 μ m long, with 7-10 germinative cells surrounded by an often clearly visible epithelium, somatic cells differentiate into three groups (towards caudal direction): primordial oviduct cells, primordial uterus cells and primordial vaginal cells, development of vaginal musculature present (Fig. 6D). J4 primordium ca 145-400 μ m long, ovary differentiated into three zones, uterus cells less granular than oviduct cells, lumen larger; two rows of three vaginal cells present, vaginal-uterus sphincter, vaginal suspensor and postvaginal cells present.

Differential diagnosis. Originally the population was mistakingly identified as Monhystera microphthalma de Man, 1880 (now Monhystrella m.: Jacobs (1987): see list of synonyms). D. dievengatensis sp. n. resembles Diplolaimella schneideri Timm, 1952 as described by Chitwood & Murphy (1964). However several differences exist: in the female, a postvaginal uterine sac is absent; in the male, several rows of germinal cells occur posterior to the growth zone (Fig. 8A) instead of only one row in D. schneideri (Chitwood & Murphy, 1964: 316), and the spicules are longer (in D. dievengatensis sp. n.: 26-29 μ m; in D. schneideri: 20-23.5 μ m).

In the genus *Diplolaimella* the males of both *D. dievengatensis* sp. n. and *D. schneideri* are characterised by the uniformity, the number and the distribution (1+1+1+2 pairs) of the postcloacal genital papillae.

D. dievengatensis sp. n. is also similar to D. ocellata (Bütschli, 1874) Gerlach, 1957, D. gerlachi Pastor de Ward, 1984 and D. stagnosa Lorenzen, 1966. D. ocellata and D. gerlachi possess respectively only four pairs and three pairs of postcloacal papillae. In D. stagnosa, the posteriormost pair of postcloacal

Fig. 8. D. dievengatensis sp. n. A - lateral view of reproductive system of σ; B-C - lateral view of anterior region of σ; B - surface view; C - internal structures; D-E - optical cross sections through the spicules, anterior to gubernaculum; F - optical cross section through the gubernaculum; av.p.g.: anterio-ventral protractor of gubernaculum, cl.: cloaca, d.g.e.: ductus glandulae ejaculatorius, d.p.sp.: dorsal protractor of spicule, i.: intestine, l.r.g.: lateral retractor of gubernaculum, ld.p.sp.: latero-dorsal protractor of spicule, sp.: spicule.



papillae is more prominent than the anteriad ones. D. stagnosa and D. gerlachi are, in contrast to other Diplolaimella species which possess prominent papillae, characterised by the absence respectively of ocelli and of well developed dorsal gubernaculum apophyses.

Type locality. Dievengat, a shallow (depth about 10 cm to maximum 50 cm) polyhaline brackish-water pond, situated in a polder near the nature reserve "het Zwin" in North Western Belgium (a full description of the type locality in Heip, 1976 and Smol *et al.*, 1980).

Type material. Holotype female (slide no. 14), allotype (slide no. 15) and paratypes (slides no. 16-20) are deposited in the Nematode Collection of the Laboratorium voor Morfologie en Systematiek, Rijksuniversiteit Gent, Belgium. Other type specimens are distributed as follows: Rothamsted Experimental Station, Harpenden, Herts, England (1 male, 1 female); British Museum of Natural History, London, England (1 male, 1 female); Vakgroep Nematologie, Landbouwuniversiteit Wageningen, The Netherlands (1 male, 1 female) and U.S.D.A., Maryland, U.S.A. (1 male, 1 female). Remaining specimens are in the same collection as the holotype.

The first author acknowledges a grant from the Institute for the Encouragement of Scientific Research in Industry and Agriculture (I.W.O.N.L.) and the last author acknowledges a grant of the Environmental Program No. ENV-767B of the C.E.C.

RÉSUMÉ

Description de Diplolaimella dievengatensis sp. n. (Nematoda, Monhysteridae)

1

à

Diplolaimella dievengatensis sp. n. a été récolté dans le Dievengat, mare d'eau de mer près de Knokke, au nord-est de la Belgique. Les différents stades juvéniles et les adultes sont décrits en détail. Le mâle de *D. dievengatensis* n. sp. est caractérisé par la longueur des spicules (plus de $^{25} \mu m$), les apophyses gubernaculaires très en relief, ainsi que par la présence et la position de cinq paires de papilles génitales postcloacales identiques et très en relief; la femelle est caractérisée par la sclérotisation de la cuticule vaginale et l'absence de sac utérin postvulvaire.

REFERENCES

BARNETT, P. R. O. (1968). Distribution and ecology of harpacticoid copepods of an intertidal mudflat. Internationale Revue der gesamte Hydrobiologie und Hydrographie 53, 117-209.

Fig. 9. D. dievengatensis sp. n. Lateral view of the spicular apparatus: A - anterior third of tail, surface view; B - spicule and gubernaculum; C - musculature of spicular apparatus, sagittal view; D - cloacal region; E-F - musculature, lateral view; G - cloacal region; av.p.g.: anterioventral protractor of gubernaculum, c.r.g.: caudal retractor of gubernaculum, d.p.sp.: dorsal protractor of spicule, l.r.g.: lateral retractor of gubernaculum, Id.p.sp.: latero-dorsal protractor of spicule, p.g.a.: protractor of gubernaculum apophyse, pv.p.g.: posterio-ventral protractor of gubernaculum, r.sp.: retractor of spicule, ro.g.: rotator of gubernaculum, v.p.sp.: ventral protractor of spicule. JACOBS, VAN DE VELDE, GERAERT & VRANKEN



Fig. 10. D. dievengatensis sp. n. A-F - anterior region: A - surface view, amphid, Q; B - stoma, σ ; C - ocellus, σ ; D - ventral view of stoma, σ ; E - ampulla ventral gland, J4 σ ; F - surface view, striae of body cuticle, J4 σ ; G-K - posterior part of pharynx and cardial region, σ ; L - intestine, σ ; b1, b2: first, resp. second buccal chamber, c: cell bodies, cc: constrictor of cardia, cs: cryptospiral fovea, dn: dorsal pharyngeal gland nucleus, ec: external cardial cell, fo a: fovea amphidealis, fu a: fusus amphidealis, ic: internal cardial cell, in: intestinal nucleus, id: lumen dilatation, ls: lumen sclerotisation, nr: nerve ring, o: ocellus 'lens', pc: pericardial cell, s₁s₁. s₁n₂: anterior set ventrosublateral pharyngeal gland nuclei.



Fig. 11. SEM-photographs of *D. dievengatensis* sp. n. a-d - anterior body end of Q; c - tail tip of Q; f-g - cloacal region, spicule tip protruded, median precloacal papilla prominent; f - direction towards tail tip: down, g - direction towards tail tip: up.

- BELOGUROV, O. I. (1985). The structure and main stages of evolution of endocupola as a skeletal system of the head end in Nernatodes. *Zoologicheskii Zhurnal* 64, 359-367 (in Russian).
- BOGAERT, T., SAMOILOFF, M. S. & PERSOONE, G. (1984). Determination of the toxicity of four heavy metal compounds and three carcinogens using two marine nematode species, Monhystera microphthalma and Diplolaimelloides bruciei. pp. 21-30 in Persoone, G., Jaspers, E.
 & Claus, C. (editors): Ecological testing for the marine environment, part 1. State University of Gent, Institute for marine scientific Research, Bredene, Belgium, 772 pp.
- CHITWOOD, B. G. & MURPHY, D. G. (1964). Observations on two marine monhysterids their classification, cultivation, and behavior. *Transactions of the American microscopical Society* 83, 311-329.
- CLARK, S. A. & STONE, A. R. (1975). A simple method of preparing nematodes for scanning electron microscopy, using Spurr's low-viscosity epoxy resin. *Nematologica* 21, 156-157.
- COOMANS, A. (1979). The anterior sensilla of nematodes. Revue de Nématologie 2, 259-283.
- GERAERT, E., REUSE, C., VAN BRUSSEL, D. & VRANKEN, G. (1981). The female reproductive system in Monhystera (Nematoda, Monhysterida). Biologisch Jaarboek, Dodonaea 49, 148-154.
- HEIP, C. (1976). The life-cycle of Cyprideis torosa (Crustacea, Ostracoda). Oecologia 24, 229-245.
- HEIP, C., HERMAN, P. M. J., SMOL, N., VAN BRUSSEL, D. & VRANKEN, G. (1985a). Energy flow through the meiobenthos. pp. 11-40. In: Heip, C. & Polk, Ph. (editors): *Concerted actions* in oceanography. Final report 3: Biological progress and translocation. Ministry of scientific Policy, Brussels, Belgium.
- HEIP, C., VINCX, M. & VRANKEN, G. (1985b). The ecology of marine nematodes. Oceanography and Marine Biology an Annual Review 23, 399-489.
- JACOBS, L. J. (1987). A checklist of the Monhysteridae (Nematoda, Monhysterida). Publ. Rand Afr. Univ., Johannesburg, 186 pp.
- LORENZEN, S. (1966). Diagnosen einer freilebender Nematoden von der schleswigholsteinischen Westküste. Veröffentlichungen des Institut für Meeresforschung in Bremerhaven 10, 31-48.
- SAMOILOFF, M. R. & BOGAERT, T. (1984). The use of nematodes in marine ecotoxicology. pp. 407-425. In: Persoone, G., Jaspers, E. & Claus, C. (editors): Ecological testing for the marine environment, part 1. State University of Gent, Institute for marine scientific Research, Bredene, Belgium, 722 pp.
- SMOL, N., HEIP, C. & GOVAERT, M. (1980). The life-cycle of Oncholaimus oxyuris (Nematoda) in its habitat. Annales de la Société Royale zoologique de Belgique 110, 87-103.
- SPURR, A. R. (1969). A low-viscosity epoxy resin embedding medium for electron microscopy. Journal of Ultrastructure Research 26, 31-43.
- VAN DE VELDE, M. C. & COOMANS, A. (1988). Ultrastructure of the photoreceptor of Diplolaimella sp. (Nematoda). Tissue & Cell 20, 421-429.
- VAN DE VELDE, M. C. & COOMANS, A. (1989). A putative new hydrostatic skeletal function for the epidermis in Monhysterids (Nematoda). *Tissue & Cell* 21, 525-533.
- VAN DE VELDE, M. C. & COOMANS, A. (1990). Ultrastructure of the anterior intestine in Monhysterids (Nematoda). Annales de la Société royale zoologique de Belgique 119, 109-119.
- VAN DE VELDE, M. C. & COOMANS, A. (in press). Ultrastructure of the buccal cavity in Monhysterids (Nematoda). Revue de Nématologie.
- VRANKEN, G. (1987). An autoecological study of free-living marine nematodes. Academiat Analecta. – Mededelingen van de Koninklijke Academie, Klasse Wetenschappen 49, 71-97.
- VRANKEN, C. & HEIP, C. (1985). Aspects of the life-cycle of marine free-living nematodes. pp. 267-278. In: Van Grieken, R. & Wollast, R. (editors): Progress in Belgian Oceanographic Research. Belgian Academy of Sciences & Belgian Center of Oceanography, Antwerp & Brussels, Belgium.
- VRANKEN, G. & HEIP, C. (1986a). Toxicity of copper, mercury and lead to a marine nematode. Marine Pollution Bulletin 17, 453-457.
- VRANKEN, G. & HEIP, C. (1986b). The reproductivity of marine nematodes. Ophelia 26, 429-442.
- VRANKEN, G., HERMAN, P. M. J., VINCX, M. & HEIP, C. (1986). A re-evaluation of marine nematode productivity. *Hydrobiologia* 135, 193-196.
- VRANKEN, G., VAN BRUSSEL, D., VANDERHAEGHEN, R. & HEIP, C. (1984a). Research on the development of a standardized ecotoxicological test on marine nematodes. I. Culturing

conditions and criteria for two monhysterids Monhystera disjuncta and Monhystera microphthalma. pp. 159-184. In: Persoone, G., Jaspers, E. & Claus, C. (editors): Ecological testing for the marine environment, part 2. State University of Gent, Institute for marine scientific Research, Bredene, Belgium, 580 pp.

- VRANKEN, G., VANDERHAEGHEN, R. & HEIP, C. (1985). Toxicity of cadmium to free-living marine and brackish water nematodes (Monhystera microphthalma, Monhystera disjuncta, Pellioditis marina). Diseases of aquatic Organisms 1, 49-58.
- VRANKEN, G., VANDERHAEGHEN, R., VAN BRUSSEL, D., HEIP, C. & HERMANS, D. (1984b). The toxicity of mercury on the free-living marine nematode Monhystera disjuncta Bastian, 1865. pp. 271-291. In: Persoone, G., Jaspers, E. & Claus, C. (editors): Ecological testing for the marine environment, part 2. State University of Gent, Institute for marine scientific Research, Bredene, Belgium, 580 pp.

Accepted for publication: September 1, 1989.