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NOTES ON MICROTURBELLARIA FROM FRESHWATER HABITATS IN THE NETHERLANDS

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INTRODUCTION

Freshwater microturbellaria of the Netherlands were studied already by De Man (1874). Since then this subject has not received much attention and much faunistic research remains to be done on this large and interesting group.

The present paper deals with material from distribution mains for drinking water in the South and the West of the Netherlands and from a canal in the city of Leiden. A new species of *Microdalyellia* is described.

MATERIAL AND METHODS

The material was taken from the following samples:

Sample no. 150. Leiden, Zoeterwoudse Singel (canal), near Koepoortsbrug. Between vegetation (*Glyceria*); decaying vegetable debris, mainly *Glyceria* and *Aesculus* leaves; 10-15 cm deep; temperature above 5° C (there was no ice in the second half of January). 20 January 1965. Leg. J. van der Land.

Sample no. 151. Same locality. 0-10 cm deep (waterlevel had fallen); temperature 4.5-5.5° C (no ice). 12 February 1965.

Sample no. 152. Same locality. 0-10 cm deep; temperature 3° C (whole canal covered with ice). 3 March 1965.

Sample no. 155. Same locality. 10-15 cm deep; temperature abt. 17°C; material strongly decomposing. 10 May 1965.

Sample no. 159. Zundert (province of Noord-Brabant); distribution mains for drinking water. Detritus sieved with plankton net from water taken from fire hydrant. I December 1964. Sample received from Waterleidingmaatschappij West Noord-Brabant.

Sample no. 161. Hillegom (province of Zuid-Holland). Same biotope. 14 January 1965. Leg. J. C. van der Vlugt. Sample no. 162. Delft (province of Zuid-Holland). Same biotope. 17 December 1964. Leg. J. C. van der Vlugt.

The animals from the drinking water could be taken direct out of the detritus when put in a dish, because these samples were rather clean, but the samples from the canal had to be treated otherwise. The vegetable matter was put in a glass cylinder and then covered with a thick layer of clean, dry sand. The cylinder was subsequently filled with water and after three to seven days the uppermost layer of the sand, in which the animals were concentrated, was washed out. In this way the animals can be obtained free from detritus.

Of the two species of *Stenostomum* I also received material from Mr. J. C. van der Vlugt of the Rijksinstituut voor Drinkwatervoorziening. He had cultivated them on agar for $1\frac{1}{2}$ month, which is a sufficiently long time to obtain large numbers. He started with animals that had been taken from sample no. 162.

Below all species are listed that were found in the samples mentioned above. In this way an impression can be given of the biocoenosis in which the described Turbellaria occur. Quantitative aspects are neglected here. Not all species have been identified. Part of these will be treated later, others are unidentifiable, e.g., because the animals were immature.

Sample nos. 150-152, 155. — Turbellaria (Stenostomum unicolor Schmidt, Catenula confusa Nuttycombe, Gieysztoria macrovariata (Weise), Microdalyellia macrobursalis n.sp., one species of Typhloplanoidea); Nematoda (not yet identified); Rotatoria (Rotaria rotatoria (Pallas), Rotaria neptunia (Ehrenberg), Dicranophorus forcipatus (O. F. Müller)); Oligochaeta (Aeolosoma hemprichi Ehrenberg, Chaetogaster diastrophus (Gruithuisen), Pristina longiseta (Ehrenberg), Nais spec., Achaeta spec., unidentifiable species of Enchytraeidae, Tubificidae and Lumbriculidae); Hirudinea (Glossiphonia heteroclita (Linné)); Tardigrada (Hypsibius spec.); Cladocera (ephippia only); Copepoda (one species of Cyclopidae and one species of Canthocamptidae); Isopoda (Asellus aquaticus (Linné)); Acari (one species); Diptera (Chironomidae larvae); Bryozoa (Plumatella repens (Linné), statoblasts only).

Sample no. 159. — Hydrozoa (*Craspedacusta sowerbii* Lankester, polyps); Turbellaria (*Stenostomum leucops* (Dugès)); Nematoda (not yet identified); Oligochaeta (*Aeolosoma niveum* Leydig, *Nais communis* Piguet, one species of Enchytraeidae); Tardigrada (*Macrobiotus macronyx* Dujardin); Cladocera (*Pleuroxus* spec.); Copepoda (one species of Cyclopidae and one species of Canthocamptidae).

Sample no. 161. — Turbellaria (Stenostomum leucops (Dugès)); Nema-

236



Fig. 1-2. Catenula confusa Nuttycombe. 1, five-zooid chain, dorsal view; 2, head region, lateral view.

Fig. 3-5. Stenostomum leucops (Dugès). 3, two-zooid chain; 4, light-refracting organ;

Fig. 6-8. Stenostomum unicolor Schmidt. 6, three-zooid chain, dorsal view; 7, light-refracting organ (Leiden); 8, light-refracting organs (Delft).

toda (not yet identified); Oligochaeta (*Nais communis* Piguet, one species of Enchytraeidae); Cladocera (one species); Copepoda (one species of Cyclopidae).

Sample no. 162. — Hydrozoa (Craspedacusta sowerbii Lankester, polyps); Turbellaria (Stenostomum unicolor Schmidt, Stenostomum leucops (Dugès); Nematoda (not yet identified); Rotatoria (Rotaria rotatoria (Pallas)); Oligochaeta (Aeolosoma leidyi Cragin, Aeolosoma hemprichi Ehrenberg, Nais elinguis O. F. Müller, Nais communis Piguet, Pristina foreli (Piguet), Chaetogaster diastrophus (Gruithuisen)); Tardigrada (Macrobiotus macronyx Dujardin).

The species of Turbellaria were studied only from living material. They can easily be studied with an oil-immersion objective by pressing them under a cover glass that rests on vaseline. Only some permanent mounts were made, as fixation is nearly always unsatisfactory. The slides are in the collection of the Rijksmuseum van Natuurlijke Historie, Leiden (abbreviated to RMNH).

Descriptions of the species

Catenulida — Catenulidae

Catenula confusa Nuttycombe, 1956 (fig. 1-2)

Catenula lemnae (pro parte?) — Marcus, 1945a: 11-13; Marcus, 1945b: 1-74; Luther, 1960: 23-24.

Catenula confusa — Jones, 1959: 335-336.

Material: Numerous specimens from samples nos. 150-152 (Leiden); some specimens from sample no. 155 (Leiden).

TABLE I

Catenula confusa Nuttycombe. Measurements in microns of three 5-zooid chains.

	Living specimen	Fixed specimen	Fixed specimen
Zooid I, cephalic region	02	511de 110. 3077 70	62
post-oral part	140	233	105
cephalic ratio	0.40	0.23	0.37
Zooid II	75	88	70
Zooid III, cephalic region	35	28	35
post-oral part	157	158	147
Zooid IV, cephalic region	62	32	45
post-oral part	150	150	112
Zooid V	75	130	115
Total length	786	889	689
Maximum width	60	70	80

Slides: Two five-zooid chains, whole mounts, sample no. 150 (Leiden), RMNH slides nos. 3077-3078.

Description. — The chains are thread-like in appearance. Length of one zooid 200-300 μ , width about 50 μ . Small chains of 0.50-1.25 mm are common, but longer chains (up to 2 mm) also occur. Very long chains were not found, but as the animals were caught with a planktonnet it is quite probable that such chains would have been broken. Chains of 5 zooids measured from 0.75 to 1.25 mm. Detailed measurements are given in table 1.

The animals are white in reflected light, but often with a brownish tinge. The cilia are fine, except for those in and near the pre-oral groove.

The length of the cephalic region is approximately twice its width or slightly smaller. The ratio of the length of the cephalic region to the length of the first zooid is 0.25 to 0.40. The lateral cephalic lobes are not very distinct; the anterior part is pointed (fig. 1-2) or rounded.

The mouth is bounded anteriorly by the pre-oral groove. The tubular pharynx makes an angle of 90° . The statocyst lies dorsally in the posterior part of the cephalic region.

The animals can swim both forward and backward and when they swim even long chains are perfectly straight.

Sexually mature specimens were not observed.

Discussion. - Nuttycombe (1956) gave an important contribution to our knowledge of the genus Catenula. In his opinion the name C. lemnae Dugès should only be used for what he called the "balustrade form" and he described material of the "non-balustrade form" that has often been confused with it as a new species C. confusa. The present material is certainly of the nonbalustrade form, but it is not completely identical with the material from the United States. According to Nuttycombe the ratio of the length of the cephalic region to the length of the first zooid is an important character. In his C. lemnae material this ratio was about 0.50, while it is 0.12 to 0.20 in C. confusa. In my material this ratio is 0.25 to 0.40 and in the C. lemnae(?) material of Marcus (1945a) and Luther (1960) this ratio is just as low or even lower (it is to be regretted that Luther did not know Nuttycombe's paper). It is possible that we do not yet know all the species of the C. lemnae group and that more than one species has the non-balustrade form. However, much more material from different localities has to be studied before we can arrive at a definite conclusion.

In sample no. 155 *Catenula confusa* was the only turbellarian species present. Luther (1960) also found it on localitites with strongly decomposing material.

Catenulida — Stenostomidae

Stenostomum leucops (Dugès, 1828) (fig. 3-5)

Stenostomum tenuicauda von Graff, 1913: 28-29.

Stenostomum tenuicaudatum Nuttycombe & Waters, 1938: 221-227, 235-237.

Stenostomum leucops leucops — Luther, 1960: 29-35.

Material: Numerous specimens from sample no. 159 (Zundert); some specimens from sample no. 161 (Hillegom); some specimens from sample no. 162 (Delft); the material from Delft was cultivated on agar so that many specimens were obtained.

Slides: 6 specimens, whole mounts, sample no. 159 (Zundert), RMNH slides nos. 3072-3073.

Description. — Only single specimens or two-zooid chains were found. Length of single zooids 600-800 μ , chains 1200-1300 μ , maximum width about 140 μ . The animals are extremely contractile but when they are swimming (fig. 3) the body is spindle-shaped, rounded anteriorly, tapering posteriorly into a long or short tail (depending on the age). Large ciliated pits lie halfway between the mouth and the anterior tip. The colour is white in reflected light. Many rod-shaped rhabdites (fig. 5) are present.

The long anterior lobes of the brain lie close to the epidermis of the ciliated pits. The posterior lobes bear light-refracting organs that are composed of more than 30 spherical corpuscules (fig. 4).

The mouth is subject to continuous changes in its shape The pharynx is three times as long as its width and does not reach the middle of the body. It is surrounded by a large number of small glands. The pore of the protonephridium lies about halfway between the end of the intestine and the tip of the tail.

Most animals were not sexually mature but one specimen contained a large, brown, oval egg on the ventral side of zooid II. When the animal was put under a coverglass the egg was discharged through the body wall, which was immediately closed again.

The animals swim rather rapidly and in a straight line, infrequently rotating about their axis.

Discussion. — This species can easily be recognized by its peculiar lightrefracting organs. Sometimes these are invisible because of the densely placed rhabdites, but when the animal is squeezed they can easily be studied.

Stenostomum unicolor Schmidt, 1848 (fig. 6-9)

Stenostomum unicolor unicolor — Luther, 1960: 39-41. Stenostomum unicolor constrictum Luther, 1960: 41-43.

Material: Numerous specimens from samples nos. 150-152 (Leiden); some specimens from sample no 162 (Delft); the material from Delft was cultivated on agar so that many specimens were obtained.

Description (material from Leiden). — Chains of two or three zooids. Length 400-1000 μ , maximum width 70 μ . The body has its maximum width



Fig. 9. Stenostomum unicolor Schmidt, head region, dorsal view (Delft). Fig. 10-11, Microdalyellia macrobursalis n.sp., habitus. 10, immature specimen; 11, $\delta + \Im$ -mature specimen.

in the pharynx region and tapers towards the blunt posterior tip. The head region is constricted just in front of the posterior lobes of the brain, the anterior part being shaped like a pointed arch (fig. 6). Colour white in reflecting light. The epidermis is thick (abt. 7 μ) and bears cilia that are less than 7 μ long. Tactile hairs are present on the whole body, 12-20 μ long.

The anterior lobes of the brain do not touch each other. They reach the posterior edges of the rather small ciliary pits. Before the brain four or five nerve knots are present. The light-refracting organs are spherical (diameter about 2.5 μ) with an excavation on the anterior side. Only in one specimen a small globulus was present before this concavity.

The mouth has a variable form. The pharynx is slender, more than three times as long as wide, with many very small glands. The post-pharyngeal region is nearly completely filled by the intestine. The intestine has two rows of excretophores with granular contents (diameter 10-12 μ). The pore of the protonephridium lies terminal.

Sexually mature specimens were not observed.

Cultivated material from Delft. — Only single specimens and small twozooid chains were found; length 300-500 μ , maximum width 50-70 μ . The cephalic region is not so strongly constricted and not pointed anteriorly (fig. 9). Not so many tactile hairs are present as in the Leiden specimens; they are up to 12 μ long. Before the anterior lobes of the brain three or four series of small nerve knots, separated by septae, are present. The lightrefracting organs (diameter about 2 μ), have an anterior depression with one or two small globules (0.3-0.5 μ) before it (fig. 8).

Discussion. — In Luther's opinion (1960) *Stenostomum unicolor* is a species complex but he was not able to divide this complex into separate species. He just described two forms.

The present material from Leiden has much in common with what he called the nominate form, but it has a constricted cephalic region. The material from Delft has more in common with Luther's forma *constrictum*. It is quite well possible that *Stenostomum unicolor* s. lat. will have to be divided into two or more species but this will not be very easy because of the lack of good characters, the great variability, and the rarity of sexual reproduction.

Neorhabdocoelida — Dalyelliidae

Microdalyellia macrobursalis n. sp. (fig. 10-22)

Material: Numerous specimens from sample no. 150 (Leiden).

242

Slides: I specimen, whole mount, glycerine, RMNH slide no. 3076 (holotype); 3 cuticular apparatus and bursa copulatrix, glycerine, RMNH slide no. 3074 (paratype I); 3 cuticular apparatus, lactic acid, RMNH slide no. 3075 (paratype 2).

Description. — Length 0.6 mm (δ -mature) — 1.5 mm (δ + Q-mature). The general shape of the body is the same as that of most species of the genus (fig. 10-11). The length is approximately $4\frac{1}{2}$ times its width when the animal is swimming. The lateral sides run parallel in small specimens; in



Fig. 12-13. Microdalyellia macrobursalis n.sp., organisation. 12, immature specimen; 13. &-mature specimen.

fully mature specimens the third quarter of the body becomes broader. The tail ends in a number of papillae. The colour is grey in reflected light but under the microscope a fine, reddish-brown pigment becomes visible, which, although present in the whole body, is concentrated in the brain-region. The cilia are small, $1-2 \mu$ long. The rod-shaped rhabdites are placed in groups of up to six. When the animal grows older the number of rhabdite cells increases more rapidly than the surface of the epidermis. Tactile hairs are present on the anterior end and on the tip of the tail. The reniform, black eyes (up to 15μ) lie closer to the lateral sides than to each other.

The digestive and excretory systems seem to be of the normal type. They were not studied thoroughly.

Male reproductive system. The mature testes lie lateral to the pharynx and the intestine (fig. 13), but in sexually immature specimens they are found in the posterior part of the body (fig. 12). The distal parts of the vasa deferentia can serve as a sperm reservoir (false vesicula seminalis). They enter the vesicula seminalis at the same place (fig. 19).

The copulatory organ lies on the left side. The organ is composed of vesicula seminalis, vesicula granulorum, penis, and cuticular apparatus. The vesicula seminalis is already filled with sperma in an early stage of development (fig. 17). It measures about 40 \times 30 μ . Later it becomes much larger (fig. 18-10) and measures for instance $85 \times 65 \mu$. The vesicula granulorum also becomes much larger in the course of its development (fig. 17-19). At first the granulae are arranged in rows, later these rows are not visible anymore. The penis is a complicated structure, the anatomy of which is not at all clear (fig. 17-19). It includes at least two ducts (ductus ejaculatorius and ductus granulorum), gland cells, and a secretion reservoir. The top is composed of some large cells. The whole organ grows equally rapid as the proximal stalks of the cuticular apparatus. I believe that good systematic characters can be found in the structure of the penis, but it has not often been adequately described so it has not yet much diagnostic value. In Gieysztoria macrovariata the penis appeared to be strongly different from that of the present species.

The cuticular apparatus is of the same type as that of *Microdalyellia rossi* (Graff, 1911) and related forms. It is composed of two proximal stalks, two distal branches with a number of spines, a connecting piece, a ventral gutter and a dorsal gutter. The stalks are not present in young specimens when the apparatus is not yet functional (fig. 12). In the course of development they constantly grow until they are even longer than the distal branches

(fig. 17-20) and meanwhile they also become broader. Their length is up to 100 $\mu.$ The length of the distal branches varies from 55 to 75 μ (in an



Fig. 14-16. *Microdyella macrobursalis* n.sp., bursa copulatrix. 14, immature; 15, mature; 16, mature, with spermatheca.

immature specimen the length was 42μ); they do not grow in mature specimens. The number of spines varies from 8 to 11. The following combinations were found: 8 + 9, 9 + 10, 9 + 11, 10 + 10, and 10 + 11. In most cases the number is not the same in both stalks. Their length decreases distally, the last spine being much smaller than the penultimate, for instance 10 μ to 18 μ , 7 μ to 14 μ . Their bases touch each other, except for the last. The first is slightly curved, the last is sickle-shaped. The ventral gutter is triangular, acute, in most cases slightly shorter than the distal branches. The dorsal gutter (fig. 21) has parallel sides in the basal two-thirds. The top consists of thin material and ends up in about six points of 3 to 5 μ length. It is about as long as the distal branches or longer. The distal points are delicate and sometimes difficult to observe.

The spermotozoon is thread-like (fig. 22). The "head" is slightly curved, about 22 μ long and up to 0.5 μ in diameter. It bears a long tail and close to its top two fine flagellae of about 20 μ long.

Female reproductive system. The bursa copulatrix (fig. 13-16) is very large and consists of two parts: the bulbus bursae ("Blase") and the vagina bursae ("Stiel"). The bulbus has a rather thin muscular wall (about 1.5 μ); its diameter is 50 to 80 μ . The vagina bursae has a thick muscular wall, up to 12 μ thick; this wall consists of longitudinal and circular muscles and of an inner membrane. The membrane, which is a basal membrane of an epithelium in its origin (fig. 14), is warty on its inner surface. The vagina is extremely contractile and constantly changes its shape (the figures represent the most common shape when the organ is relaxed). In a number of specimens the bursa contained a spermatheca filled with sperma (fig. 13, 16). The bulbous part of this theca (diameter about 50 μ) is situated in the bulbus bursae, the shorter or longer neck projects into the vagina bursae. Sometimes free spermatozoa are present in the vagina. It was once observed that a group of spermatozoa was moved from the vagina into the uterus and back.

In small specimens the vitellaria are smooth (fig. 11) but later papillae develop (fig. 13). The germarium is situated on the right side of the body (fig. 13). A short, thick germiduct is present, measuring about $40 \times 40 \mu$. The receptaculum seminis is not separated from the germiduct. It can be filled already with sperma in small specimens. Egg-bearing specimens were not observed.

In the intestine were found setae of Oligochaeta (Nais spec.) and trophi of Rotatoria (Rotaria rotatoria and Dicranophorus forcipatus).

Discussion. — The structure of the cuticular apparatus indicates that the present species is closely related to *Microdalyellia rossi* (Graff, 1911). It is possible that material of the present species was included by later authors

246

in the latter species, but we can not be sure about this because the animals were often inadequately described. Most important differential characters can be found in the arrangement of the cuticular spines, the structure of the dorsal gutter and the bursa copulatrix (the latter was observed to be extremely small in M. rossi (cf. Luther, 1955)).

The \circ cuticular apparatus acts as a whole but in fact it consists of two parts that are formed of different material and that behave different in



Fig. 17-18. Microdalyellia macrobursalis n.sp., & copulatory organ in two stages of development.

248 ZOOLOGISCHE MEDEDELINGEN 40 (1965)

ontogenesis: a distal part (gutters, distal branches and spines) and a proximal part (stalks and connecting piece). The distal part is not striated, it is not affected by KOH and it does not grow in mature specimens. The proximal part has a striated, fibrous appearance and it disappears when it is treated with KOH; in young specimens it is not present at all and in \Im -mature specimens it grows continuously. Attention should be paid to these facts when the male apparatus is described. The inner membrane of the vagina bursae



Fig. 19-22. Microdalyellia macrobursalis n.sp. 19, 3 copulatory organ; 20, cuticular apparatus; 21, dorsal gutter of cuticular apparatus: 22, spermatid.

(which is in fact the \bigcirc cuticular apparatus) is not affected by KOH either so it possibly consists of the same material as the distal part of the \Diamond cuticular apparatus. It is not impossible that the latter in its origin is also a structure of a basal membrane.

Ruebush & Hayes (1939) discussed the relationship within the genus Dalyellia (including the subgenus Microdalyellia). In their opinion the position of the testes is important, the anterior position of the same being a primitive character. Species in which the testes are located in the posterior portion of the body are most highly evolved. It is interesting that in the present species the testes have a posterior position in juvenile specimens (fig. 12) and that they later move to the anterior part of the body. Luther (1955) observed in Microdalyellia rossi that in Q-mature specimens, the reduced testes are located again in the posterior part. We should be inclined to conclude from this that the posterior position is primitive and not the anterior position as was supposed by Ruebush & Hayes who partly base their phylogenetical theories on this assumption.

Gieysztoria macrovariata (Weise, 1942) (fig. 23-28)

Dalyellia rubra var. macrovariata Weise, 1942: 145, 160-165, 183. Gieysztoria macrovariata subsp. novemspinosa Luther, 1955: 209-212.

Material: A few specimens from sample no. 150 (Leiden); 1 juvenile specimen from sample no. 152 (Leiden).

Description. — Length 360 μ (immature specimens) — 600 μ (δ -mature specimens); Q-mature specimens were not found. In immature specimens the tail ends in a number of papillae (fig. 24). The epidermis is thick (approximately 12 μ) and the cilia are long (12 μ). The eyes are reniform, the posterior lobe being the largest; they measure from 14 to 18 μ . The length of the pharynx is 1/4 to 1/5 of the body length.

Male reproductive system. The testes lie for the greater part posterior to the intestine (fig. 23). The total length of the δ copulatory apparatus is 165 to 205 μ . The penis is a rather simple structure (fig. 25), with a bulbous base surrounded by the vesicula granulorum. The distal part was only slightly longer than the cingulum of the cuticular apparatus but perhaps the penis was not yet full-grown. The cuticular apparatus has a cingulum ("Gürtel") which has no fenestrae and which is up to 42 μ long. Its membrane is reinforced by a distal ring (proximal ring not present), by fibrous basal extensions of the spines and by a network of small fibres. The 10 spines are symmetrically arranged, the central pair being the shortest (about 45 μ from the distal ring), the lateral pair the longest (75-90 μ).

Female reproductive system. Only the bursa copulatrix was studied (fig.



Fig. 23-28. Gieysstoria macrovariata (Weise). 23, habitus; 24, tail of juvenile specimen; 25, 3 copulatory organ; 26, five spines of the cuticular apparatus (flattened and treated with KOH); 27, bursa copulatrix, extended condition; 28, bursa copulatrix, same specimen, contracted.

27-28). It is very contractile and is composed of a long vagina and a large bulbus (up to 75 μ in diameter). The proximal part of the vagina bursae has a thick epithelium in which groups of needles are embedded, just as they were observed by Luther (1955) in part of his material of the subsp. *novemspinosa*. In Luther's specimens they were about 10 μ long but in the present material they measure only 4 to 6 μ . The needles are arranged in star-shaped groups (diameter 8.5-11.5 μ). Seven groups were observed in one vagina.

Discussion. — This species has only been found in Berlin (Germany) and in Pisa (Italy) so it is apparently not a common species. The present material conforms very well to the original description. In the material from Pisa one central spine and eight lateral spines were present in the cuticular apparatus. In my opinion this is not a reason to consider it a separate subspecies. In the original material from Berlin specimens with 9 spines also occurred.

When the cuticular apparatus was treated with KOH the greater part of the cingulum disappeared (fig. 26; in this figure the distal ring has been drawn although it also disappeared nearly completely). It appears that the basal parts of the spines reinforce only a small part of the cingulum. The cingulum is apparently homologous with the proximal stalks and the connecting piece in the cuticular apparatus of *Microdalyellia*.

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