

## Further studies on the occurrence of freshwater Microturbellaria in the British Isles

### I. A description of *Macrostomum johni* sp. nov.

JOHNSTONE O. YOUNG *Department of Zoology, The University, Liverpool*

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#### Summary

*Macrostomum johni* sp. nov. from Llyn Cwellyn, N. Wales is described. The species has been recorded from the vegetational littoral in December and February only. The main distinguishing criterion is the penis stylet which consists of a funnel, about 80–98  $\mu\text{m}$  in length in slightly squashed specimens, curved in two planes and with a sub-terminal distal opening, the distal end is blunt and cowl (hood) shaped. Other features include the presence of sensory hairs and rhabdoids all over the body, 'Haft-papillen', and eyes. Rhammite tracks are weakly developed. The testes are elongate and the ovaries are compact, though slightly indented, and smaller than the testes. A very short ductus intervesicularis is present. The vagina is short. A 'Verschlussapparat' is present but poorly developed.

#### Introduction

Rhabdocoels of the Family Macrostomidae have been studied in some detail, for example, by Beklemishev (1951), Ferguson (1939–40, 1954), Luther (1947, 1960), Marcus (1952, 1954), and Papi (1951, 1953, 1959). Papi (1967) lists the freshwater species of *Macrostomum* found in Europe. This paper describes a new freshwater species of the genus *Macrostomum* which was found during an investigation of the occurrence of Microturbellaria in some British lakes (Young, in press; in prep.). The specific name *johni* ascribed to the new species is a tribute to the author's late father who first and continually stimulated his interest in natural history.

#### Methods

Specimens were obtained using an F.B.A. zooplankton net of 60 meshes/in. (23.6 meshes/cm) mounted in a square frame with a 5-ft (1.52-m) pole. The net was used to sweep through vegetation and to scoop up the surface layers of substrata. The samples were left to stagnate in 3 litre glass jars in the laboratory, and the animals were removed at intervals (see Young, 1970).

Anatomical studies were based upon living and preserved whole mounts, squash preparations and on paraffin-wax sections of individuals killed in Steinmann's fluid,

Correspondence: Dr J. O. Young, Department of Zoology, Brownlow Street, P.O. Box 147, Liverpool, L69 3BX, England.

fixed in a saturated mercuric chloride/acetic acid solution (100 ml/5 ml), and cleared in cedar-wood oil. The specimens were sectioned at 8  $\mu\text{m}$  thickness, stained in Ehrlich's haematoxylin and eosin and mounted in Canada balsam. Permanent whole mounts, unstained or stained in borax-carmin, and squash preparations were mounted in Canada balsam or polyvinyl lactophenol.

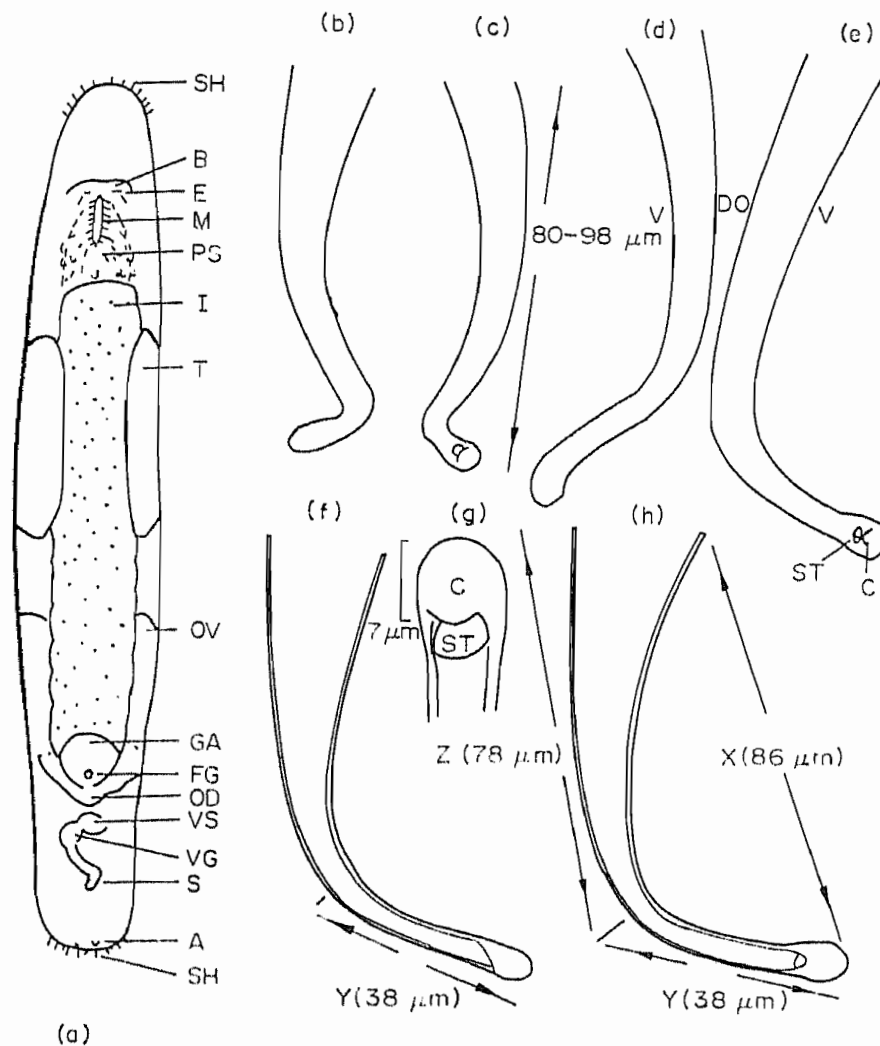
### Habitat

*Macrostomum johni* sp. nov. was recorded from the littoral zone of the east shore of Llyn Cwellyn, Caernarvonshire, N. Wales (O.S. Ref. 23560550) in December 1968, February 1969 and February 1970. Monthly samples taken from November 1968 to October 1969 (August excepted) did not reveal any animals outside these 2 months. Though samples were taken from stones, 'typical' substrata without vegetation, and beds of vegetation (*Agropyron repens* (L.) Beauv., *Juncus subnodulosus* Schrank, *Littorella lacustris* L. and *Phalaris arundinacea* L.), only the latter revealed any specimens. Indeed most specimens were recorded from the *P. arundinacea* zone which usually occurred in the deeper water (1–1.5 m at normal water level) in the littoral vegetation. Altogether some fifty specimens were obtained. Some of the physico-chemical features of Llyn Cwellyn appear in Reynoldson (1958) and Young (in preparation).

### Description of species

The description is based upon a syntypic series; whole organisms and stained sections are deposited as type specimens with the British Museum (Natural History), London; Reg. Nos. 1972.3.13.1., 1972.3.13.2., 1972.3.13.3. Lengths of up to 1.3 mm for sexually mature animals have been recorded. The body is more or less parallel sided though it tapers slightly towards the anterior and posterior extremities and there is a slight constriction or waist in the region of the penis stylet; the middle is fractionally wider than the rest of the body and is about 1/5th of the body length (Fig. 1a). The anterior and posterior extremities are somewhat rounded. The body is colourless though the enteron has a yellowish colour in transmitted light; the enteron varies in colour however according to its contents. The epidermis consists of cells, 4–10  $\mu\text{m}$  in height and tallest in the anterior tip of the body, resting on a weakly developed basal membrane; mucous cells are also scattered in the epidermis. A dense coat of cilia, about 4–8  $\mu\text{m}$  long, covers the entire external body surface. Sensory hairs up to 25  $\mu\text{m}$  in length but usually smaller (12–20  $\mu\text{m}$ ), are sparsely scattered in tufts over the body; there are up to six hairs in each tuft but on average two to four. At the anterior and posterior ends the sensory hairs are more abundant, especially at the posterior end; they are usually solitary but on occasion in smaller bundles; the anterior hairs on occasion appear to be stiffer than the posterior hairs but probably do not equate with the so-called semi-rigid sensory spines found in some *Macrostomum* species.

Rhabdites are scattered all over the body in bunches of up to nine though four to six are common; their distribution perhaps is slightly less dense at the anterior end; their approximate length is about 9–15  $\mu\text{m}$ , though rhabdites up to 18  $\mu\text{m}$  have occasionally been observed particularly in the tail region. 'Stabchenstrassen' or rhammite tracks which in some *Macrostomum* species develop in the neighbourhood of the 'brain' and send their ducts forward to empty at the anterior end of the body, are present but weakly developed in this species; indeed in many specimens they were scarcely apparent. 'Haftpapillen', epidermal outgrowths from the posterior end of the body, were present and averaged 8–10  $\mu\text{m}$  in length.



**Fig. 1.** (a) Drawing of *Macrostomum johni* sp. nov. to show the various anatomical features (ventral view). (b, c, d and e) Free hand drawings of unsquashed penis stylet in ventral, dorsal, side and side view respectively (in side views the dorsal (DO) and ventral (V) surfaces are indicated). (f, g and h) Camera lucida drawings of stylet in squash preparations: (f) and (h) show entire stylet and (g) the distal tip (cowl or hood) only; distances X, Y and Z are shown as averages (see text for extremes) for fully mature animals. A, 'Haftpapillen'; B, brain or cerebral ganglia; C, cowl or hood; E, dark eyes; FG, female gonopore; GA, genital atrium; I, intestine or enteron; M, mouth; OD, oviduct; OV, ovary; PS, simple pharynx; S, penis stylet; SH, sensory hairs; ST, sub-terminal opening; T, testes; VG, vesicula granulorum; VS, vesicula seminalis.

The nervous system is fairly typical for that described for other species of *Macrostomum*. The brain is crescent-shaped and composed of two ganglia connected by a short commissure, which in effect is represented by a slight indentation in the mid-line of the cephalic neural masses. In a dorsal view of living, sexually mature animals of about 1.2 mm, the antero-posterior width of the ganglion in the eye region was  $32\ \mu\text{m}$  whilst this width of the brain through the commissure in the mid-line was  $27\ \mu\text{m}$ . The brain consists of a central matrix of fibrous tissue surrounded by large cell bodies. Smaller anterior and stout lateral longitudinal nerves, which extend back along the length of the body, arise from the brain, which is situated equidistant from the dorsal and ventral sides of the body. The eyes are positioned dorso-posterior to the ganglia of the brain and appear to be embedded in the tissues of the brain. The dimensions of the eye in mature individuals varied and lengths of  $10\text{--}15\ \mu\text{m}$  and breadths of  $5\text{--}7.5\ \mu\text{m}$  were observed. The number and size of the component granules varied;

extremes of fifteen to sixty granules were counted, and the largest granules measured  $2.2\text{--}2.7\ \mu\text{m}$ . The distance between the eyes is less than between the eye and the lateral margins of the body.

The alimentary canal is typical of that found in other *Macrostomum* species and consists of a mouth, pharynx simplex and enteron (blind gut, enteric sac or intestine). Muscle fibres extend posteriorly and laterally from the pharynx. Pharyngeal glands are present and are found from the level of the anterior margin of the enteron and are most abundant laterally. The enteron has weakly undulant walls and the tall enteric epithelium is provided with cilia about  $6\text{--}10\ \mu\text{m}$  long. Inner circular and outer thicker longitudinal fibres comprise the musculature of the enteron. Radiating fibres extend from the walls of the enteron to the body-wall but the distribution of these has not been studied in detail.

Indeed the musculature of the animals has not been worked out in detail as yet. Refined histological details will be described in a future paper. Suffice to say that interior to the epidermis inner longitudinal and outer circular and occasionally inner diagonal muscle fibres have been observed and their degree of development varies from location to location in the body. Dorso-ventral muscles have also been observed.

The excretory system, normally difficult to see, again has not been studied in detail at present. Certainly it is bilateral, as usual in species of *Macrostomum*, and comprised of two main coiled stems with branching excretory ducts which terminate in flame cells. The two main lateral protonephridial tubes almost meet in the mid-line just in front of the brain. Cross stems between the lateral tubes have not been observed but may or may not be present. Nor have external openings been found as yet.

The basic reproductive system is typical of that found in other *Macrostomum* species. Thus the female reproductive system comprises paired ovaries and oviducts, a common oviduct, a genital atrium, a female gonopore and its accessory glands. The paired ovaries are slightly indented and lie in a latero-dorsal position. Their size varies according to the state of sexual maturity, however, they are usually little over half as long as the testes (see Fig. 1a). In living specimens they usually do not extend forwards to meet the testes but in histological preparations shrinkage causes them almost invariably to meet. The oviducts, usually fairly indistinct in the absence of eggs, extend latero-caudally from each ovary and caudally, in the region of the genital atrium, bend to join in the mid-line at the posterior margin of the atrium. The genital atrium again varies in size according to the state of sexual maturity. A 'Verschlussapparat' is present but poorly developed and in quite a few specimens it was not observed. The enteron just overlaps, dorsally, the genital atrium in living specimens (Fig. 1a) but extends behind the atrium in sectioned material. A short 'vagina' extends obliquely ventro-caudally to the gonopore. Cells containing granular secretions open into the vagina.

The male reproductive system is typically composed of paired testes and vasa deferentia, a vesicula seminalis, a vesicula granulorum, a penis (stylet), and a male gonopore. The testes are elongate (Fig. 1a) and situated latero-ventrally. They stretch from just behind the anterior end of the enteron to approximately half to two-thirds the way along the body in a fully mature animal. The size of the testes depends on the size and state of maturity of an animal; lengths of between  $210\ \mu\text{m}$  and  $345\ \mu\text{m}$  were recorded in living animals. The breadth of a testis in an animal with a testis of length of  $294\ \mu\text{m}$  was  $71\ \mu\text{m}$ . The surface of the testes is fairly smooth. A vas deferens situated mid-way between the body wall and the enteron extends from the posterior

tip of each testis caudally until it reaches the posterior end of the enteron where it bends to join its counterpart in the mid-line of the body; at this point, in fully mature animals a false vesicula seminalis is formed, and the size of this varies according to the state of maturity. The false vesicula seminalis opens via a slight constriction into the vesicula seminalis which in turn opens via a very short ductus intervesicularis, supplied with very few muscle fibres, into the vesicula granulorum. The walls of the vesicula seminalis and vesicula granulorum have spiral muscles. Granule-producing cells are located close to the vesicula granulorum at the penis stylet. Again, the size of the vesicula seminalis and vesicula granulorum depends on the state of sexual maturity of the animal and both are capable of distension.

The penis stylet consists of a funnel, curved in two planes (Fig. 1b, c, d, e), which has its base connected with the vesicula granulorum and its tip located near the male gonopore. The distal termination has the form of a slightly swollen cowl or hood with a thickened wall, and with a sub-terminal opening on the concave side of the cowl (Fig. 1c, e, f, g, h; Plate 1a, b, c). In squash preparations the stylet usually appears as a tube curved in one plane only at an angle in excess of  $90^\circ$  (Fig. 1f, h; Plate 1a). The second bend in the funnel is not apparent as it has been squashed flat. The size of the stylet varies according to the state of maturity. In apparently fully mature animals of size 0.9–1.3 mm the following extreme measurements X, Y and Z (see Fig. 1h) have been recorded in squash preparations: X, direct distance from proximal opening to distal tip, 78–95  $\mu\text{m}$ ; Y, distal tip to bend, 30–45  $\mu\text{m}$ ; Z, bend to proximal opening, 70–85  $\mu\text{m}$ . In slightly squashed apparently fully mature animals of length 0.9–1.3 mm, in which the curves in the two planes were still obvious, the length of the stylet from proximal to distal ends varied from 80–98  $\mu\text{m}$  (Fig. 1c). The proximal opening in the squashed specimens varied between 19–33  $\mu\text{m}$ . The cowl length, usually equal to breadth, in a stylet in which measurement X was 85  $\mu\text{m}$  was 7  $\mu\text{m}$  (see Plate 1c).

To give some indication of the position and size of some of the various anatomical structures length measurements from a living sexually mature individual of approximately 1.3 mm in length, and selected at random from several similar specimens, will be quoted: anterior tip to eyes—147  $\mu\text{m}$ ; eyes to posterior tip of mouth—74  $\mu\text{m}$ ; posterior tip of mouth to beginning of enteron—70  $\mu\text{m}$ ; beginning of enteron to anterior tip of testes—70  $\mu\text{m}$ ; testes length—320  $\mu\text{m}$ ; posterior tip of testes to anterior tip of ovaries—100  $\mu\text{m}$ ; anterior tip of ovaries to posterior end of female atrium—240  $\mu\text{m}$ ; and posterior margin of atrium to posterior tip of body—260  $\mu\text{m}$ .

### Discussion

Till now only two freshwater species of *Macrostomum* have been recorded in natural conditions in the British Isles (Young, 1970): *M. distinguendum* (Papi, 1951) and *M. rostratum* (Papi, 1951). Young & Young (1967) recorded *M. tuba* (Graff, 1882) from British freshwater aquaria. The discovery of *M. johni* sp. nov. in Llyn Cwellyn boosts the number of naturally occurring freshwater species of *Macrostomum* in Britain to three.

The shape of the penis stylet is alone regarded as sufficient a basis for the separation of *M. johni* sp. nov. from all other *Macrostomum* species. Indeed apart from this single feature, no further morphological characteristic enables *M. johni* sp. nov. to be distinguished from all the other members of the genus.

*M. johni* is probably related to *M. lutheri* Beklemishev 1927 (see description in Papi, 1951) the stylet of which is much longer and curved in one plane only. The

stylet tip of *M. johni* sp. nov. looks similar to that *M. mediterraneum* Ax 1956 (see description in Ax, 1959); however, the stylet of this marine species is nearly straight.

### Diagnosis

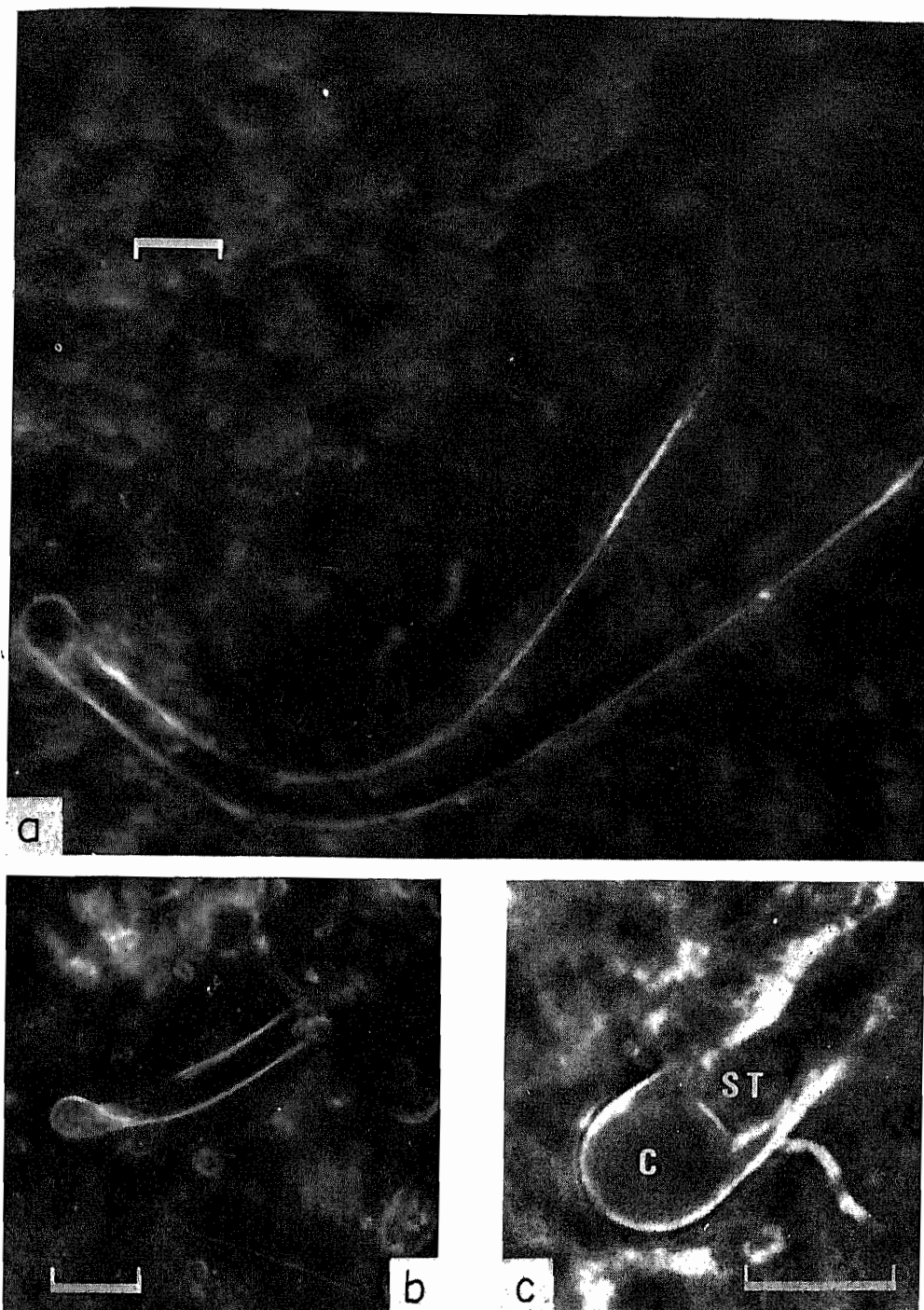
Length up to 1.3 mm. Sensory hairs and rhabdoids all over body. 'Haftpapillen' present. Rhammite tracks ('Stäbchenstrassen') weakly developed. Eyes present. Elongate testes. Very short ductus intervesicularis present. Penis stylet curved in two planes and 80–98  $\mu$ m in length; distal end blunt and cowl (hood) shaped, and the distal opening sub-terminal; proximal opening 25–33  $\mu$ m. Ovaries shorter than testes, compact and only slightly indented. Vagina short. 'Verschlussapparat' poorly developed.

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**Plate 1.** Penis stylets of *Macrostomum johnei* sp. nov. photographed *in situ* from squash preparations of living material. (a) Fully developed stylet. Scale = 10  $\mu$ m. (b) Developing stylet in animal not fully mature. Scale = 9  $\mu$ m. (c) Distal tip of fully developed stylet to show cowl and sub-terminal opening. Scale = 7  $\mu$ m. C, cowl or hood; ST, sub-terminal opening.