

MACROSTOMUM HUSTEDI, N. SP.; A MORPHOLOGICAL AND
CYTOLOGICAL STUDY OF A RHABDOCOEL
TURBELLARIAN

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TEN TEXT FIGURES AND ONE PLATE (FOUR FIGURES)

INTRODUCTION

In August and September of the years 1940, 1941, and 1942, the tide-pools of Duxbury Bay, Massachusetts, U. S. A., abounded with a pigmented rhabdocoel. This animal was plainly the dominant form among the bottom-dwelling turbellarian fauna, being at least thrice as numerous in collections as the common *Monocelis lineatum* von Graff. Under the compound microscope the animal was easily studied, being rather transparent in spite of the pigmented epithelial cells that covered certain of the dorsal regions. The animal was recognized as belonging to the genus *Macrostomum* Schmidt. Its striking pigmentation (plate, fig. 4) being unlike that of any reported species (according to Ferguson, '38, et. seq.), it was thought to belong to a new species. Many of these *Macrostoma* were therefore fixed for special study. Such study revealed that the new species has a chromosome number twice that of most other species whose number has been determined, and thrice that of two of the reported species of *Macrostomum*. The possibility that the new species might be a polyploid thus lent interest to the study of the anatomy and cytology of *Macrostomum hustedi*, n. sp.¹

PART I: SYSTEMATICS AND MORPHOLOGY

Living animals were immobilized by pressure of a vaseline-sealed and supported coverglass. Sketches of living forms, and of the more obvious organs, were made, for later comparison with fixed material. Animals were crushed, while under observation, and the structure of the stylet, the ciliated epidermal and endodermal cells, and the spermatozoa could be studied. Other animals were fixed in Goldsmith, Strong Fleming, San Felice, Carnoy, or La Cour's 2 BE. Sections for morphologi-

¹ Named in honor of Dr. Ladley Husted, of the University of Virginia, who has generously aided and instructed the author in this and in other cytological research.

cal studies were made 8μ thick. Total mounts were made, either stained or unstained. Sections were stained with iron haematoxylin, decolorized in picric acid, and counterstained with eosin or acid fuchsin.

Description

Macrostomum hustedi is a medium-sized macrostomum, showing considerable variation in adult length. Of sixteen specimens fixed by the same method, and all with a mature or nearly mature ovum in the female genital atrium, the average length was $750 \pm 110\mu$. The generically distinctive "streamlined" or teardrop shape (marred only by the adhesive tail) is characteristic of the new species. The appearance of the eyes, the distribution of cilia, sensory hairs, and packets of rhabdites, or small rhabdite-glands, also follow the generic pattern, in that the animal in these respects resembles one or another of the described species of *Macrostomum*.

The pigmented epidermis is a specifically distinctive character. Figure 4, of the plate, shows the pattern of pigment upon the dorsal surface. The anterior third of the animal is colored a rich gold-brown by the pigment. Clear spaces extend dorsal and lateral to the eyes. This pigment becomes very scattered posteriorly and upon the ventral surface. Details of the pigment granules, as they appear in section, are seen in the plate (plate, fig. 2). The granules themselves appear in life less dense than the pigment of the eye cup. Their color is brown, as opposed to the latter's black. They are less closely packed. Their location and appearance do not suggest a glandular function. That their function may be accessory to the visual function is suggested by the correspondence between their pattern of distribution and the location and assumed field of vision of the eyes. The eye itself is of the type described by Kepner and Stiff ('32) for *Macrostomum tuba*. Each eye has a single retinula, consisting of ellipsoid-, rhabdome- and myoid-elements, connected with the cephalic ganglion and a pigment cup.

The tail is a spatulate appendage connected with the body proper by a constricted region of considerable muscular strength. The adhesive faculty of the tail is achieved by a concentration of rhabdites in that region. Like most *Macrostoma*, this form freely attaches and detaches the tail to and from the substrate, using it as a base upon which to move the body about, or as an anchor against too swift a current.

The internal anatomy is not distinctive. The enteron is a simple sac, without diverticula or indentation. Its epithelium consists of irregular columnoid cells with long cilia or flagella. In the enteron wall are smaller

generalized cells. The male genital system consists of a curved stylet, directed posteriad (plate, fig. 3), a granular portion weakly supplied with prostatic gland cells, a small vas deferens or seminal vesicle, and two vasa efferentia leading from the two subspherical testes, which lie on either side in the oesophageal region. The spermatozoa, in both fresh and fixed preparations, appear filiform, without flagella or setae. The female genital system consists of the vagina, with its surrounding mass of glandular tissue, the common oviduct (here serving the combined functions of oviduct and atrium, since no histological separation can be made between such regions), and the two oviducts leading from the lateral ovo-vitellaria.²

Unlike the freshwater *Macrostoma*, the new species produces eggs with very thin shells. In the common oviduct or genital atrium, where the eggs undergo maturation, the shell substance is seen in the form of scattered peripheral granules. Perhaps the extensive glandular tissue of the vagina adds its secretion to this shell substance at the time of deposition.

Macrostomum hustedi n. sp.: Specific Diagnosis. Rhabdocoeles of the family Macrostomidae Bresslau, 1932, genus *Macrostomum* Schmidt, 1848, with characteristics of the genus. Length 750 ± 110 micra; shape "tear-drop," with blunt anterior end and slender tail. Color white, with brown pigment on dorsal surface of anterior third of body; pigment separated to form clear areas dorso-lateral to eyes. Ciliation uniform, without ciliated pits. Sensory hairs uniformly distributed, except for ventral surface, somewhat concentrated at anterior terminal region, scarce to absent on ventral surface. Body sparsely covered with a single kind of compound rhabdite or small rhabdite gland; these rhabdites concentrated on tail surfaces, scarce on ventral surface of body. Short "Haft-papillen". Male and female systems in general as found elsewhere in the genus. Stylet has orifice about one-third distance from point to base. Sperm motile in water, filiform, naked. Female genital canal equipped near orifice with mass of gland-cells. Common oviduct

² Since, in this animal, the ovary shows histological resemblance to the ovo-vitellaria or germaria of the cumulate alloecocoeles, it is suggested that this organ has a dual, or compound function as producer and nourisher of ova. Meixner's ('23) view that "indifferent germ cells" in *Macrostomum* yield their nutritive content, by cellular disintegration, to the support of the oocytes finds no corroboration in the present observations. By structural analogy to the entolecithal eggs of certain cumulate alloecocoeles, as well as to those of the Hymenolepidid cestodes, it seems probable that the ovary of *M. hustedi* secretes nutritive yolk material, which is absorbed by the developing oocytes. Kepner and Stiff ('32) are of the opinion that in *Macrostomum tuba* no cellular disintegration occurs, but that all ovarian cells are "ova-potential." Whether or not the ovary of *M. hustedi* actually has a secretory function has not been determined.

indistinguishable from female genital atrium. Ovary serves as oovitelarium. Chromosome number $2n = 12$. Habitat, mud-flats of Duxbury Bay, Massachusetts. Type specimens: U. S. National Museum. Paratypes: Author's collection at Southwestern University, Georgetown, Texas.

Discussion

The taxonomic position of the new species is probably close to that of *Macrostomum beaufortensis* Ferguson, 1937 and not far removed from that of *Macrostomum appendiculatum* (O. Fabricius) Ferguson, 1938.

M. beaufortensis, like the present species a marine form, was reported (Ferguson, '37) as having a "Light brown epidermis supplied with small reddish brown inclusions. There is a broad band of these epidermal inclusions located just anterior to the mouth on the ventral side, and two broad bands lateral to the mouth. These inclusions do not show well in reflected light." While it is possible that these "inclusions" may be similar to the pigment granules found in the new species, neither their distribution nor their appearance (except color) suggests identity of the two. Moreover, Ferguson's *M. beaufortensis* is twice as large as *M. hustedi*, contains zoochlorellae in its parenchyma, has rhabditenstrassen in the anterior region, confines its sensory hairs to the posterior end of the body only, and has the chromosome number $n = 3$, $2n = 6$. While the sperm and stylet of *M. beaufortensis* are quite similar to those of the new species, the above details clearly distinguish it from the latter.

From *Macrostomum appendiculatum* the new species may likewise be easily distinguished. *M. appendiculatum* has three kinds of rhabdites; its enteron is ventral (?) to the common oviduct and ovaries; no pigmentation has been described for it; it has a discernible excretory system (not observed during careful study of living *M. hustedi*); its spermatozoa are biflagellate, not filiform and naked as in *M. hustedi*. Other species of *Macrostomum* are also different, in greater degree than the discussed species, from the form described in this paper.

PART II: CYTOLOGY

Materials and Methods

Animals fixed in the cytologically valuable fluids of San Felice, Fleming ("strong"), Carnoy, and La Cour (2BE) were dehydrated and embedded by the chloroform method suggested by La Cour ('31). Sec-

tions were cut at 10 and 14 micra. Iron haematoxylin, differentiated with picric acid, did not give as good results as Newton's crystal violet. The latter stain, after 2 BE, gave excellent results. In the thinner sections (10 micra), spermatocytes in late prophase and in metaphase I and II of meiosis could be studied critically. In the thicker sections (14 micra), the meiosis of oocytes could be studied without the extensive reconstruction necessitated by thinner sections. Drawings were made at exactly determined magnifications, with the aid of a Zeiss camera lucida.

Description

The somatic chromosome number of *Macrostomum hustedi* is twelve. Consideration of this number in relation to chromosome numbers in other species of the genus *Macrostomum* suggests, at once, polyploidy. Chromosome number determinations have been made for thirteen of the forty-odd species defined by Ferguson ('38 et seq.). Ruebush ('38) and Ferguson (by personal communication, '42) think that some of these determinations may be incorrect. Phillips' ('36) study of the chromosomes of *Macrostomum tuba* was, however, a critical work. The diploid number 6, determined by Phillips for *M. tuba*, has been reported for ten other species (Ruebush, '38; Ferguson, '38 et seq.). The number 4, reported by early workers for two species, is the only other chromosome number reported for a *Macrostomum*. Thus the number 12 reported for the present species appears to be an even multiple of either of the base numbers hitherto given; it may on the basis of number alone be a tetraploid, base 3, a triploid, base 4, or a hexaploid, base 2, or perhaps a more complex derivative from the commoner numbers, with subsequent changes in structure.

The chromosome morphology helps confirm the above suggestion of polyploidy. There are six chromosomes with submedian constrictions, and six rod-like chromosomes, with subterminal constrictions. The submedians are so close to the same size as to be morphologically indistinguishable. The rods are also close to the same size, but seem to belong to two size groups, three being larger, three smaller.³ Mitotic plates from mesenchymal tissue and from young gonads are difficult to study, being small; but the above classification and count of the chromosomes

³ The presence of two groups of three morphologically similar chromosomes thus indicated in the present study, raises problems that cannot readily be solved in this paper. If, by a series of exact measurements, the existence of two such groups can be proven, then at meiosis at least one heteromorphic bivalent must of course be expected. But small bivalents, condensed as they are (text fig. 5), are not easy to analyze; heteromorphic bivalents were often suggested but could not be recognized with certainty.

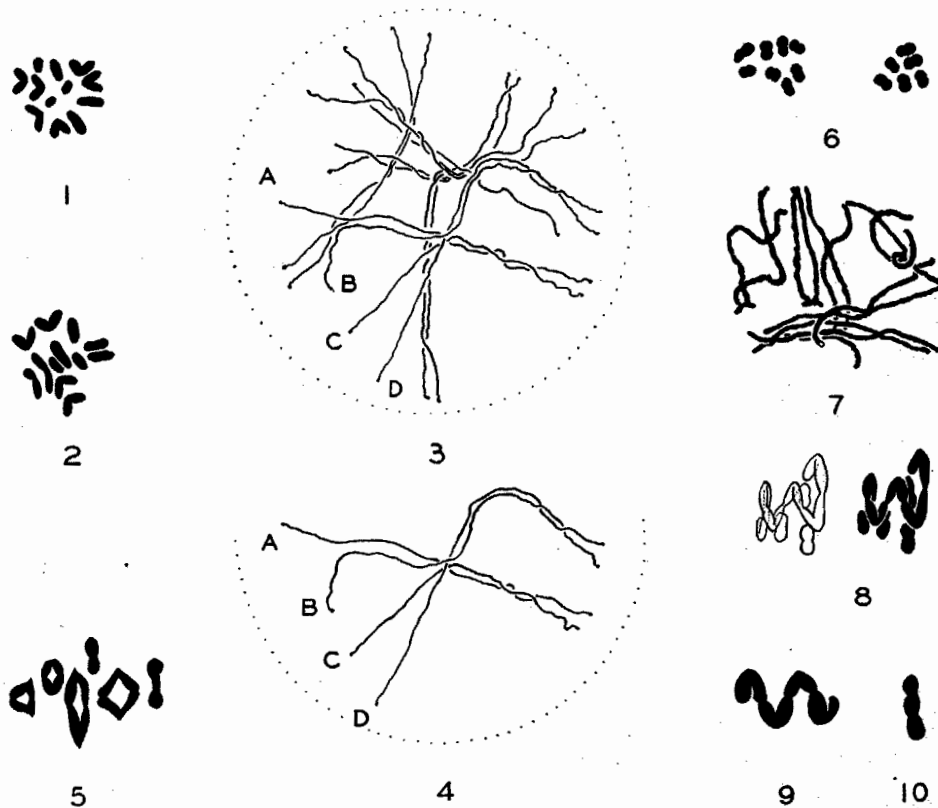
could be made from nearly all the animals studied. The chromosome morphology, therefore, by the apparent reduplication of chromosome types, gives additional evidence of polyploidy.

The meiotic behavior of the chromosomes of *M. hustedi* shows further evidence that the species is a polyploid.

In spermatogenesis, counts of chromosomes at first and second meiotic metaphase appear to conflict, in many cases. Numbers of from four to eight are common, in addition to the usual number, six. The spermatocytes are so small that critical study of bivalent structure is difficult. It is possible to see that some associations involve more than two chromosomes; but the exact nature of such association, without guiding information from other sources, is not determinable in most preparations. It appears equally probable that the chains and groups of metaphase or prometaphase chromosomes may be either true multivalents or fixation-fused groups associated at congression. In some spermatocytes, however, multivalents of a classical configuration can be recognized as such. The chain of four chromosomes, of the type shown in figure 9, and the chain of three, shown in figure 10 were seen. In the cases referred to, interpretation was based on favorable orientation and good general fixation. The probability of the configurations' being fusion-artifacts was checked against the presence, in the same cell, of distinct and discrete small bivalents lying within a critical distance of other chromosomal masses. The failure of such small groups to have fused with larger adjacent groups shows that the "fusion" within the large groups must have occurred through other causes than fixation, viz. such causes as chiasma formation between more than two chromosomes. Thus the observations upon spermatocyte meiosis tend to confirm the suggestions of polyploidy already discussed.

Oogenesis supplies more evidence. The oocyte of *M. hustedi* is relatively very large. Its prophase chromosomes fix well with 2 BE or San Felice. A critical study of zygotene, pachytene, and early diplotene can be made. True multivalent associations are found at zygotene and pachytene. Text figures 3 and 4 are illustrations of a zygotene association of four chromosomes. Other configurations were studied, but were too complex to be profitably represented. In these complex prophases the multiple associations were often obscured by mechanical crossing and touching of bivalents probably not due to true pairing of homologous regions. Thus only a few perfectly clear examples of multivalents could be recognized in oocyte prophase, although there were many complex "tangles" in which multivalent pairing almost certainly played a part.

The results of the pairing of more than two chromosomes are traceable in the later stages of meiosis. Certain of the spermatocyte first metaphases (see above and text fig. 8) can be safely interpreted as containing multivalents. Second metaphases in the oocyte (text fig. 6) show the results of non-disjunction or unequal disjunction of first metaphase



Text figures 1-10. All figures were drawn with the aid of a Zeiss camera lucida, at the magnifications given below.

Fig. 1, 2 Mitotic chromosomes, $\times 1600$.

Fig. 3 Oocyte at zygotene. A, B, C, D are four chromosomes supposedly homologous. $\times 350$.

Fig. 4 Chromosomes A, B, C, and D above drawn separately for clearness.

Fig. 5 Metaphase I of meiosis in spermatocyte, somewhat spaced for clearness. $\times 1600$.

Fig. 6 Two plates of metaphase II in oocytes, the one at the left having eight chromosomes. $\times 800$.

Fig. 7 Diplotene from an oocyte. $\times 350$.

Fig. 8 Metaphase I from a spermatocyte, drawn at the left in outline to show details of a chain association of six chromosomes. $\times 1600$.

Fig. 9 A chain of four from spermatocyte metaphase. $\times 2000$.

Fig. 10 A trivalent from spermatocyte metaphase I. $\times 2000$.

associations. Oocytes with seven and eight second metaphase chromosomes, as well as those with the usual six, were seen.

Oogenesis, as well as spermatogenesis, therefore, suggests that some form of polyploidy⁴ exists in *M. hustedii*.

Discussion

Polyploidy in animals has been discussed theoretically rather often. The view has been expressed, by Muller ('25) and White ('40), among others, that polyploidy in bisexual animals is rare, presumably because the sex mechanism and ratio in bisexual forms would be upset by polyploidy. This view is challenged by Gates ('42), with the suggestion that the *Drosophila* "balance" sex-mechanisms (upon the assumed prevalence of which among animals the above views are predicated) may not prevail among animals, and that, instead, the "Y-determining" mechanism found in *Melandrium* may be predominant. Under the latter view, sex ratios would be preserved even in high polyploids. Some cytologists (Contra: Darlington, '37, p. 223, who suggests adaptive and genetic controls as inhibiting the establishment of polyploidy among animals in general) believe that hermaphroditic and monoecious forms, on the other hand, should exhibit polyploid series just as do the higher plants.

Before attempting to evaluate the evidence supporting the present and other reports of naturally occurring polyploidy in animals, the general evidential pattern supporting inference of polyploidy should be traced.

Extensive studies of polyploidy in plants have revealed the following kinds of evidential data: First, the members of a taxonomic group may differ from each other by exact multiples of a certain "base" chromosome number. (The many "polyploid series" reported by plant cytobotanists need not be cited here, since they are so extensive. This

⁴ Speculation as to the sort of polyploidy evinced by *Macrostomum hustedii* seems not very profitable. Since haploid 3 is the commoner of the two base numbers reported in *Macrostomum*, the present form, "diploid" 12, should on that account be considered a tetraploid. The occurrence of quadrivalents supports that idea. Yet the possible divisibility (see footnote 3, p. 351) of the chromosome types into three identical sets of four is evidence of triploidy. Absence of a report of a base 4 in the genus should not per se be an objection to the idea of triploidy, since less than a third of the known species of *Macrostomum* have been cytologically investigated, and the base 4 may be present in the genus. The possibility of hexaploidy arising by doubling of the chromosomes of a spontaneous triploid (Fankhauser, '41, that triploidy is commonest form of spontaneous polyploidy) should not be dismissed (see chain of six chromosomes, text fig. 8). A more complex situation, with breakage and translocation, may be involved, as is often the case in "old" polyploids. In view of these possibilities, it cannot be stated at present what the exact condition is in this species.

evidence is, by far, the commonest type). Second, study of the morphology of the chromosomes of a suspected polyploid may reveal triplication, quadruplication, etc., of recognizable chromosomes. (This evidence includes the data reviewed by Gates, '42b, from the counts of satellite chromosomes and nucleoli). Third, the meiotic bivalents may be grouped in associations not linked by chiasmata, by a phenomenon called "secondary pairing" (Darlington, '37; Wanscher, '34). Fourth, actual multivalents may occur at meiosis, which, if the probability of translocation be reduced, may be considered strong support for an hypothesis of polyploidy otherwise suggested. (The numerous reports of known polyploidy accompanied by multivalent occurrence need not be cited here). Genetic and anatomical evidence may at times be adduced, but, in general, is secondary to the chromosomal evidence of the sorts mentioned above. Where such evidential facts are present in combination, it may often be said that a case for polyploidy has been made out.

In chromosome studies of animals, very few cases of naturally occurring polyploidy have been inferred from a combination of evidential data. Most of these reports are based on one or another of the above types of evidence, without corroborative data. Thus, while the summary of White ('40) concerns the possibility of the occurrence of a polyploid number-series in the hermaphrodite Turbellaria, Mollusca, and Hirudinea, as revealed by chromosome numbers, his conclusion that such a series may be present is as yet (before the present paper) unsupported by other types of data than the numbers themselves. In the urodele amphibians, the occasional occurrence of polyploid individuals has been reported (Fankhauser, '41). The actuality of these cases is undoubted; but in only one individual has the possibility of establishment of a polyploid line been observed. In this case (Böök, '40), the only reported case of a polyploid urodele's metamorphosis and sexual maturity, spermatogenesis was observed, and the behavior of the chromosomes of the triploid showed possibilities of typical polyploid variation in gametes. (While the animal examined was destroyed before its somatic chromosome number was determined, it seems highly probable that the animal was entirely triploid). The variation in chromosome number among different species and genera in the amphibia is generally explicable, not by partial or complete polyploidy, but by fusion-breakage phenomena. This is also true of the number variation in many of the arthropoda. (It may be noted that in his report of a trisomic orthopteran Callan, '41, explained the absence of trivalents as due to low chiasma frequency. Apparently, from other reports, multivalent association

does take place in orthopteran polyploids or polysomies. White, '33, has described multivalents from a tetraploid testis in *Locusta gregaria*. See, also, Suomalaenen, '40; Kawaguchi, '38; Gremillion, '42; Mickey, '42). The discussion of mammalian chromosome numbers by Gates ('42a), reviews in evidential form certain determinations, with the contention that they may form a polyploid series. The report of Sanderson ('40) merely adds another item of numerical-type data to the summary of White ('40).

Thus it appears that while polyploidy in animals does "naturally" occur, it does not seem to be common. And while the evidence for it in individuals is generally conclusive, the evidence for polyploidy in any species is confined to scattered reports (e. g., Sanderson, '40; Suomalaenen, '40; White, '40), in which there is little combination of the different types of data whence, in plants, inference of polyploidy may safely be drawn.

The present report, has described an animal species, apparently well established (from collections seemingly constant over three seasons), which exhibits several of the kinds of evidence for polyploidy. In a genus where all other chromosome numbers reported are four and six, diploid, the present species has twelve chromosomes. This is numerical evidence of the sort adduced by White ('40) and others. The morphology of the chromosomes shows that six are possibly identical, and that the other six are either identical or separable morphologically into two groups of three. This suggests, as Callan's ('41) evidence did, that individual chromosomes have been reduplicated in excess of simple pairs. Multivalents (like those reported by Bök, '40, in Triton) occur, as they do in some of the polyploid plants. While genetic evidence of the results of polyploidy is lacking in the present species, yet the observation of second metaphase nuclei with variable chromosome numbers implies the probability of cytologically varied gametes and is, thus, perhaps akin to genetic evidence; semisterility, such as that known in many plant polyploids, may be expected to occur in the present animal polyploid, even though the latter's predominance over the other fauna of its bionomic level indicates a successful reproductive adaptation. Breeding experiments on this rhabdocoel may yield interesting data.

SUMMARY

1. A new species, *Macrostomum hustedi*, is described. It is characterized chiefly by a pigment-pattern in the epidermis, by marine habitat, and by the chromosome number $2n = 12$. The new species is

distinguished from the species closest to it, *M. beaufortensis* and *M. appendiculatum*, by several obvious characters.

2. The chromosomes of *M. hustedi* are described and analyzed. Six V's and six rods are reported. Three items evidential of polyploidy are noted: first, the diploid number is double or triple that of other species in the genus; second, the chromosome morphology shows reduplication of chromosomes beyond simple pairs; and third, multivalents are fairly common in meiosis.

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PLATE 1

EXPLANATION OF FIGURES

Macrostomum hustedi, n. sp.

All drawings were made with the aid of a Zeiss camera lucida, and reproduced at the magnification indicated by the scales.

- 1 Reconstructed lateral view, partly in perspective, of *M. hustedi*. Labelled are: E, eye; P, pharynx; M, testis; OV, ovary; O, oöcyte; F, female genital canal (vagina); A, female accessory glands; V, vas efferens of right side; S, seminal vesicle; and ST, stylet.
- 2 Section through pigmented epithelium. Labelled are: RH, rhabdite gland or compound rhabdite; PI, pigment granules in ciliated cell.
- 3 Sagittal section through stylet and male genital pore. Labelled is: OR, the stylet orifice.
- 4 Habit sketch, dorsal view, of *M. hustedi*. Labelled is: PI, pigment pattern.

