

12.

***Trichodina spheroidesi* and *Trichodina halli* spp. nov. Parasitic
on the Gills and Skin of Marine Fishes, with Special
Reference to the Life-history of *T. spheroidesi*.**

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Plates I-III; Text-figures 1-4.

INTRODUCTION.

Marine members of the family Urceolariidae have been described from several species of invertebrates and vertebrates. Among those parasitizing fish are the following: *Trichodina scorpaena* Robin (1879) from the gills of *Scorpaena* and *Trigla*; *T. labrorum* Chatton (1910) from two species of *Symphodus*; and, *T. fariai* da Cunha & Pinto (1928) from the intestine of the smooth puffer, *Spheroides testudineus*. Fantham (1918, 1919, 1924, 1930) reported *Trichodina* from several fishes of which the following were definitely identified as new in his 1930 paper: *Trichodina clini* from *Clinus taures*, *C. superciliosus*, *C. capensis*, *C. cottoides*, *C. anguillaris*, *Pristopoma bennettii* and *Box salpa*; *T. blenni* from *Blenius cornutus*, *T. mugilis* from *Mugil capito* and *T. chelidonichthys* from *Chelidonichthys cupensis*.

Because of insufficient information on many of the above forms, it was extremely difficult to arrive at a definite conclusion as to the validity of these species. In the present studies, the writers encountered two forms of *Trichodina* parasitic on the gills and skin of puffers (*Spheroides maculatus*) and other marine fishes from the New York and New Jersey coast. These peritrich ciliates are easily distinguished from one another on absolute size and other characters. The one, a smaller and more abundant form, is designated *Trichodina spheroidesi*. The other, larger in all respects and less frequently encountered, is called *Trichodina halli*. This species can easily be distinguished from other *Trichodina* infesting

marine fishes on the basis of body size and size and relationships of the organelles (see Table).

MATERIAL AND METHODS.

Between the months of June and the early part of October, from 1938 to 1940, about three hundred of the puffers taken in pound nets in Sandy Hook Bay were examined for *Trichodina*. Two hundred and forty-eight, or 82%, of the fish were found parasitized, the intensity varying considerably.

A number of gill samples were fixed in Schaudinn's fluid without acetic acid, and others were fixed in 10% neutral formalin. The material was stained with Heidenhain's iron-hematoxylin and Mallory's triple stain. A few samples of those treated by the former method were counterstained with "light green." Only the Schaudinn-fixed specimens were found suitable for cytological studies. Studies on the adhesive disc and denticulate ring were made from both the Schaudinn and formalin fixed material.

A combination of the Klein's (1926) silver impregnation and the De Fano's reduction techniques was used to determine the ciliary pattern. Especially good results for such studies were obtained with material fixed with formalin. The samples were washed in five changes of distilled water, impregnated with a 5% solution of silver nitrate for a period of eight to twelve hours, and placed in darkness. Following the removal of excess silver nitrate solution the material was reduced in De Fano's solution. The films were toned in a 3% solution of sodium hydrosulphite and sodium anthraquinone sulphonate (25:1). This method,

although not delicate enough to give a distinct silver line system, nevertheless effectively demonstrated the ciliary pattern, including the basal granules.

Measurements were taken of the height, the diameter of the organism, the diameter of the adhesive disc and of the denticulate ring. A count was made of the hook number on all mounted organisms.

HOST-PARASITE RELATIONSHIPS.

A variety of fishes indigent to the Sandy Hook area were found to harbor *Trichodina* (Nigrelli, 1940). The infestation on any one host species was never found as consistently or as intense as on the puffers. Insofar as could be determined, trichodiniasis among puffers was limited to fish on the New York and New Jersey coast, for examination of this species from the coast of Massachusetts during part of the period of this investigation did not reveal infection.

A few migratory *Trichodina* were found moving about on the body surface of puffers. However, the heaviest infestation was always on the gills. This may be attributed to the small opercular opening which encourages the concentration of these ciliates in the gill chamber.

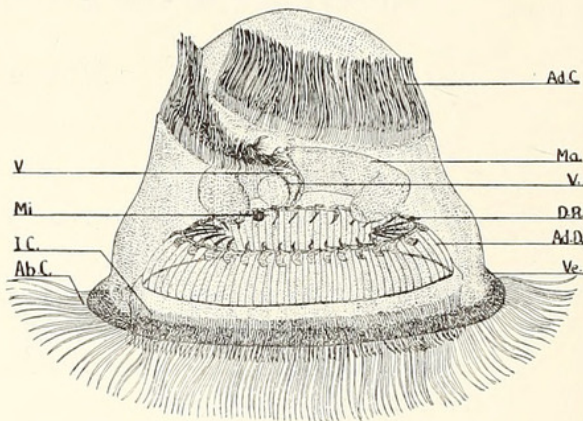
The presence of red blood cells in food vacuoles of the parasites show that they are capable of considerable tissue destruction. This is indicated further by the fact that in exceptionally heavy infestation the gill epithelium was completely destroyed, leaving large denuded areas among the filaments. Such a condition results in the death of the host.

Trichodina spheroidesi sp. nov.

(Text-fig. 1).

Description: Organisms turban shaped. Diameter ranges from 17–54 μ ; height from 12–42 μ . Two parallel rows of adoral, long, flexible, closely set, cilia present; cilia begin a short distance from the base of the peduncle, make one and a quarter clockwise, spiral turns and terminate in the vestibulum just above the mid-sectional plane of the oral surface; cilia, shortened noticeably at a point near the entrance of the vestibulum, spirals twice along the wall of the vestibulum and at the lower end are twisted together; direction of this spiral, when viewed from the end of the vestibulum outward, also clockwise. Aboral region with two rings of cilia; one ring, fused to form a membranelle, attached to inner posterior surface of the velum; second ring of cilia, found between membranelle and outer surface of the adhesive disc, are more delicate and approximately half as long as those forming the membranelle. Adhesive disc is a deep saucer-shaped organelle, ventral in position,

bordered on the dorsal side by the denticulate ring and on the posterior side by the inner row of cilia; diameter of adhesive disc ranges from 18–32 μ ; striae, present on inner and outer walls of the disc, are argentophilic. Denticulate ring non-argentophilic; diameter of the ring varies from 14–22 μ ; denticles of the ring with hooks on outer border and slender rays on inner border, joined together by triangular projections (Text-fig. 4); number of hooks varies from 21–31. Macronucleus is typically horse-shoe



Text-fig. 1. *Trichodina spheroidesi*. Side view. $\times 950$. Semi-diagrammatic reconstruction from hematoxylin and silver nitrate preparations. Ad. C., adoral cilia; V., vestibulum; Ma., macronucleus; C. V., contractile vacuole; Mi., micronucleus; D. R., denticulate ring; Ad. D., adhesive disc; Ve., velum; I. C., inner ring of aboral cilia; Ab. C., outer ring of aboral cilia.

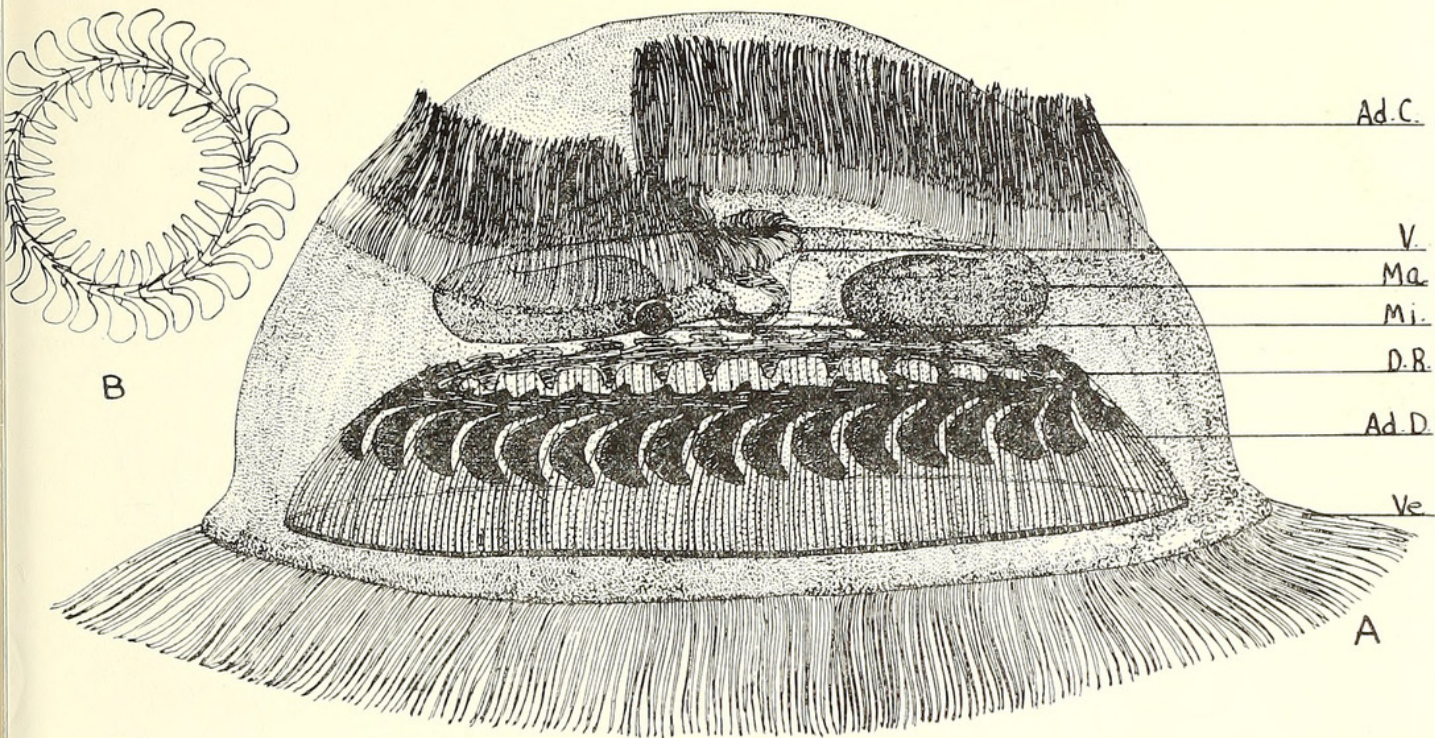
shaped, with comparatively few basophilic granules; it lies in the posterior half of the cell, parallel to the dorsal plane of the adhesive disc; the open ends of the macronucleus extend to the oral side and surround the descending portion of the gullet. Contractile vacuole lies close to the descending portion of the vestibulum and during trophic life, fission and post-conjugative reorganization, it is found in the posterior half of the cell. Trophic micronucleus small, lies close to the left tip of the macronucleus; during conjugation its position is variable.

Remarks: Many individuals of this species were found to be infected with the suctorian parasite, *Endosphaera engelmanni*. In such cases, position of the nuclei and other cytoplasmic contents may be greatly displaced. The above description is based on several hundred normal ciliates. *Trichodina spheroidesi* is distinguished from other *Trichodina* described from marine fishes by the presence of the inner ring of aboral cilia.

Trichodina halli sp. nov.

(Text-fig. 2).

Description: Hemisphere or dome-shaped organisms. Diameter, taken at the base,



Text-fig. 2. A. *Trichodina halli*. Side view. $\times 950$. Semi-diagrammatic reconstruction from hematoxylin and silver nitrate preparations. Legend same as in Text-fig. 1. Note the absence of inner aboral ring of cilia. B. Denticulate ring. $\times 475$.

ranges from 45–86 μ . Adoral cilia present, similar in arrangement and extent to *T. spheroidesi*. Only one ring of aboral cilia evident; membranelle absent. Disc diameter varies from 41–81 μ ; striae present in two layers. Denticulate ring thicker than in *T. spheroidesi*; diameter ranging from 30–54 μ ; denticles fitted together by the insertion of a cone-shaped protuberance of one denticle into a corresponding depression in adjacent one (Text-fig. 2, B). Number of hooks varies from 26–34; hooks shaped and curved like a ship's propellor blade and joined to the denticles at an angle so that when observed on edge the broad area is not evident and they appear to be crescent shaped. Macronucleus as in *T. spheroidesi*, except that arms are longer and there are more basophilic granules present. Position of the micronucleus and other structures typical.

Remarks: *Trichodina halli* can be distinguished from *T. spheroidesi* in the following ways: (1) size of organism and organelles considerably larger, (2) ratio of disc diameter to the diameter of the organism as a whole is less, (3) denticulate ring thicker, (4) shape and arrangement of denticles and hooks entirely different, (5) longer arms of the macronucleus, and (6) lack of inner ring of aboral cilia.

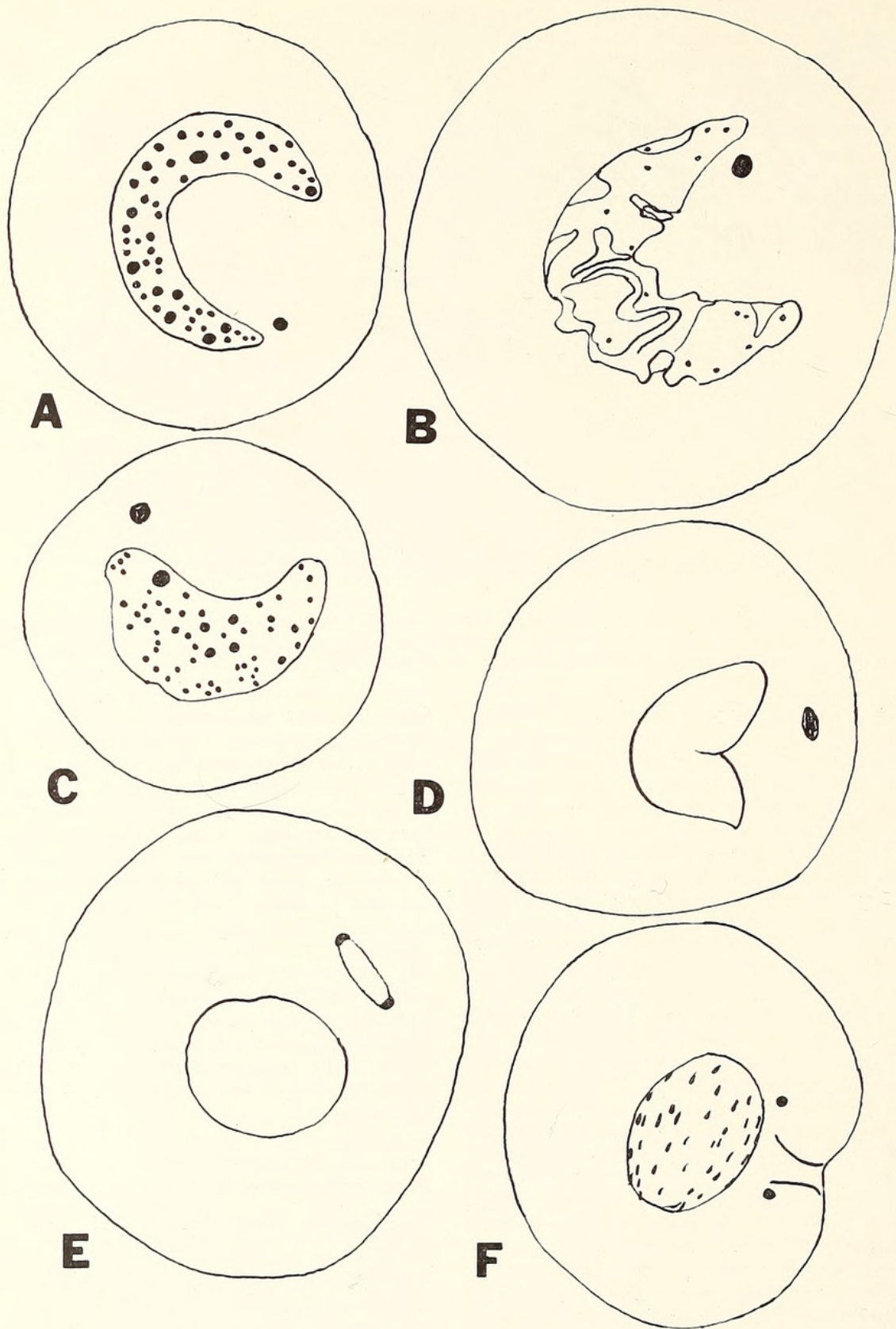
The variation in size of both *T. spheroidesi* and *T. halli* suggests that other species of *Trichodina* reported in recent years may have presented a like variation in range of

measurement if sufficient numbers were studied. A review of the reported species shows that in very few instances was this range adequately determined. In the table below a comparison of measurements of various structures of *T. spheroidesi* and *T. halli* with the available data of some previously reported marine species is made. As may be seen from this table all the species, except *T. halli*, fall within the size range of *T. spheroidesi*. The distinguishing features of most of these forms consist mainly in host specificity (?) and minor variations as to shape, ciliary pattern, nature of adhesive disc and denticulate ring. Since such information is not given in sufficient detail these organisms cannot be keyed out.

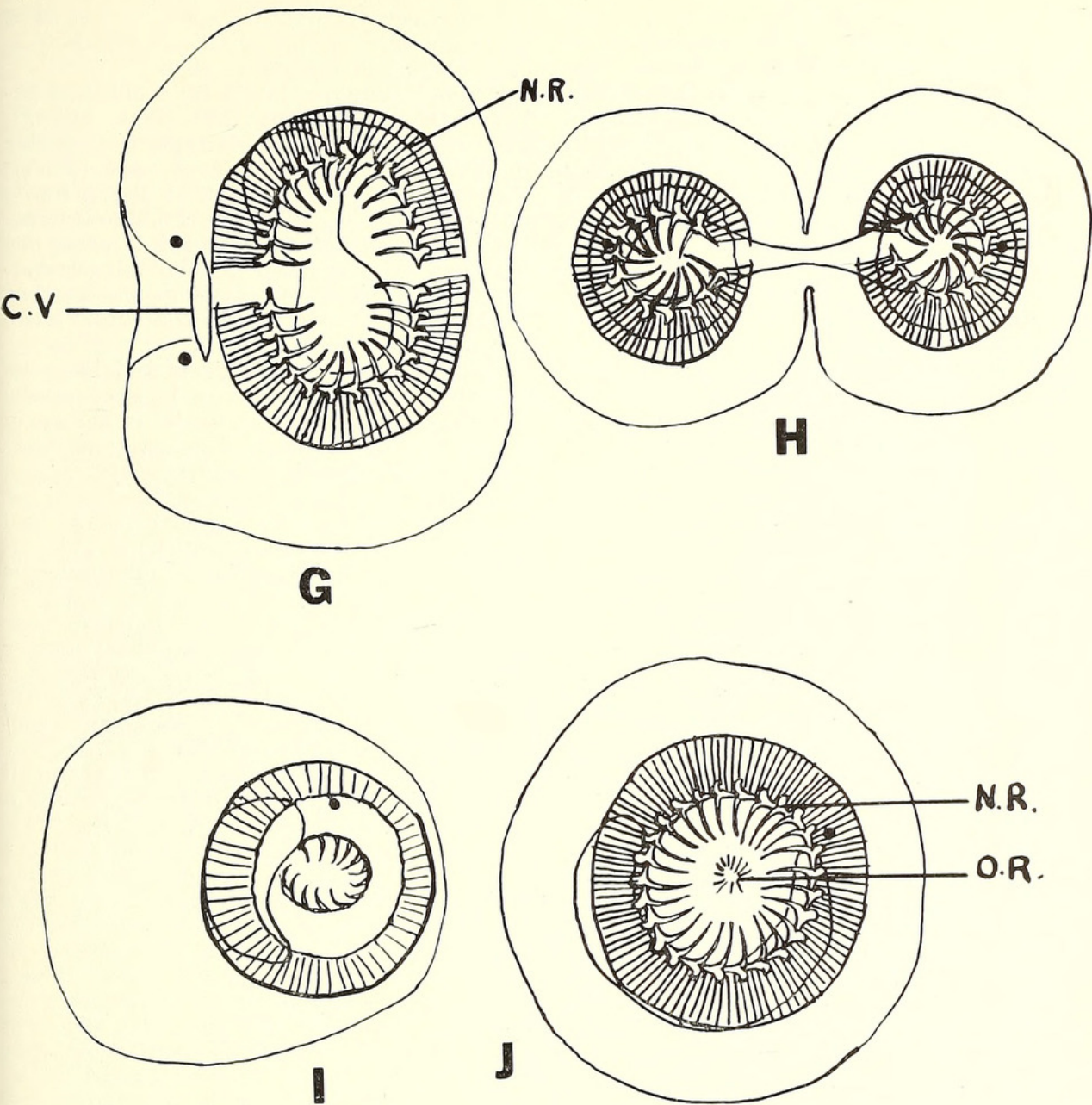
TABLE I.					
Measurements (in mm.) for <i>Trichodina</i> from Marine Fishes.					
Species	Height	Width	Disc Dia.	Ring Dia.	Hook No.
<i>T. blenni</i>	20–32	40–45	24–27		24–32
<i>T. clini</i>	20	37	20		24
<i>T. chelidonichthys</i>	19–27	30–45	19–32		30
<i>T. mugilis</i>	14–20	33–44	23–28		32
<i>T. fariai</i>	32	40		20–42	24–28
<i>T. labrorum</i>	18–22	30–34			21
<i>T. spheroidesi</i>	12–42	17–54	18–32	14–22	21–31
<i>T. halli</i>		45–86	41–81	30–54	26–34

BINARY FISSION IN *Trichodina spheroidesi*.
(Text-figs. 3 & 4).

The stages of fission in *T. spheroidesi* correspond very closely to those described by Diller (1928) for the species of *Tricho-*



Text-fig. 3. Binary fission in *Trichodina spheroidesi*. $\times 950$. From iron-hematoxylin preparations. A, trophic stage; B, macronucleus in stage of contraction; C, D, and E, further contraction of the macronucleus; B and C, note swelling of micronucleus; D, micronucleus in metaphase; E, telophase; F, completed micronuclear division.



Text-fig. 4. *Trichodina spheroidesi*. $\times 950$. Iron-hematoxylin preparations. G, H, I, J, binary fission stages showing the final division of the macronucleus and the reorganization of the denticulate ring. C. V., contractile vacuole; N. R., new ring; O. R., old ring.

dina from tadpoles. The trophomacronucleus undergoes vacuolization and clefts appear in the ground substance (Text-fig. 3, B). The macronucleus begins to condense while the chromatin granules, large in the trophic stage, become smaller in size and randomly distributed in the matrix. As the macronucleus contracts further, the micronucleus swells, eventually becoming spheroidal, then divides mitotically (Text-fig. 3, C, D, E). A metaphase stage of a dividing micronucleus is shown in Text-fig. 3 D. The mitotic division continues as the macronucleus pulls apart, and two daughter micronuclei are formed before the macronucleus is com-

pletely divided (Text-fig. 3, F; Text-fig. 4, G). Plasmotomy takes place about the time of the late telophase. A stricture appears in the ventral portion of the organism, and the adhesive disc and denticulate ring separate into approximately equal halves (Text-fig. 4, G, H). Final cleavage of the macronucleus takes place and two daughter cells are formed (Text-fig. 4, H). Peshkovsky (1923) reported that during division of *Trichodina steinii* and *T. mitra* the adoral cilia, gullet and contractile vacuole are absorbed. Careful study of the silver impregnated material, however, leads the

writers to believe that the adoral and the aboral zones of cilia, in *T. spheroidesi* at least, are retained throughout division. The fate of the vestibulum and the vestibular cilia could not be traced. In specimens stained with hematoxylin, it was observed that the contractile vacuole is retained also during cell division. It appears to cleave concomitantly with the cleavage of the macronucleus (Text-fig. 4, G).

The anlage of the new denticulate ring (corona) is laid down as a delicate ring close to the posterior border of the adhesive disc before the onset of cell division (Text-fig. 4, G). This confirms the observations of Diller (1928). After the formation of the daughter cells the new corona gradually develops its denticles and hooks and takes up a more dorsal position on the adhesive disc. The rays are the last structure to be formed in the ring. Half of the old ring, carried over after cell division by each daughter cell, is pushed into the ventral plug of the cytoplasm between the aperture of the adhesive disc. Here it is slowly resorbed (Text-fig. 4, I, J).

CONJUGATION IN *Trichodina spheroidesi*.

(Pl. I-III).

Conjugation in *T. spheroidesi* is anisogamous and the process is very similar to that reported by Maupas (1888) for *Vorticella nebulifera* (see also Doflein, 1927). The aboral surface of the microconjugant is fitted over the adoral surface of the macroconjugant (Pls. I, II). They may or may not be oriented in the same direction. After the conjugants have assumed their respective positions, the micronucleus of each begins to swell, eventually becomes vesicular and passes from the original posterior position to a more central location in the cell (Pl. I, Figs. 1-3). The macronucleus begins to show signs of vacuolization (Pl. I, Fig. 2).

At the time of spindle formation, the macronucleus twists and pulls apart into large coarse fragments (Pl. I, Fig. 3). These pieces continue to break up into smaller and smaller parts until minute spherical bodies with deeply staining granules are formed (Pl. I, Figs. 4, 5, 6, 7, 8; Pl. II, Fig. 9).

Micronuclear activity immediately preceding the metaphase period is not clearly defined (Pl. I, Fig. 4). It is possible that the micronuclei have a decreased affinity for iron-hematoxylin stain during these stages. The metaphase spindle, however, is clear and granular chromosomes can be identified about the center (Pl. I, Fig. 5). During the final fragmentation of the macronucleus, two micronuclear divisions take place in each conjugant (Pl. II, Fig. 10).

It is of interest to point out here that conjugation in *T. spheroidesi* differs from

conjugation in *Vorticella nebulifera* in that there are only two micronuclear divisions in each conjugant instead of three.

Protoplasmic continuity is established between the conjugating individuals (Pl. II, Fig. 11), and the contents from the smaller individual pass into the larger one (Pl. II, Fig. 12). It is assumed that the gametic nuclei (Pl. II, Fig. 10) then combine to form the synkaryon, and the remaining nuclei are resorbed. The remains of the microconjugant collapse and the ensuing processes of conjugation are confined to the single large exconjugant (Pl. II, Fig. 13).

At the onset of its first division, the zygotic nucleus develops a larger spindle than any of the dividing nuclei in the early stages of conjugation. It is assumed that three mitotic divisions subsequently occur, resulting in eight micronuclei. Seven of these become the macronuclear anlage and one the functional micronucleus (Pl. II, Figs. 14, 15). The functional micronucleus divides (Pl. II, Fig. 15) and in the cell division which takes place, the macronuclear anlage are distributed between the daughter individuals (Pl. III). The most frequent distribution is three and four (Pl. III, Figs. 16, 17). However, the distribution may sometimes be two and five (Pl. III, Figs. 18, 19) or one and six. Cell division continues until each of the daughter cells formed contain one macronuclear anlage (Pl. III, Fig. 20). The macronucleus then increases in size and develops its characteristic horse-shoe shape.

Reorganization of the denticulate ring occurs in the macroconjugant shortly after the fusion of the protoplasmic contents of the conjugants has occurred. Figures 12 and 13 of Plate II show the newly formed ring together with the remains of the old denticulate ring. It should be pointed out that the original ring is present in preceding stages but is not shown in the figures for sake of clarity. It is of interest to note that the number of denticles in the new ring is invariably the same as the number present in the old ring. Such structures as the gullet, vacuole and cilia are present throughout conjugation.

DISCUSSION.

Trichodina spheroidesi is distinguished from other *Trichodina* of marine fishes by the presence of the inner ring of aboral cilia. Wetzel (1927) and Precht (1935) reported a similar observation for *T. pediculus* and *T. scoploplontis*, respectively.

The double layer of striae in the adhesive disc observed by the writers in the two forms from the puffers have been previously reported by Mueller (1932, 1938) for *Trichodina renicola* and *Vauchomia nephritica*. Mueller (1938) refers to the inner group of striae as the posterior hard rays and the outer group as the anterior soft

rays. He further commented that the soft rays comprise a system of myonemes which connect with the posterior girdle of cilia. This duplex nature of the striae Mueller featured to distinguish members of the Trichodinidae found in the urinary tract of fishes (e.g., *T. renicola* and *V. nephritica* from *Esox niger* and *Esox masquinongy*, respectively) from those found on the gills of certain fresh-water fishes reported by him in 1937. In the ciliates from the gills only the hard rays were present in the striated ring. However, the occurrence of double striations in the forms described by the writers suggests that this feature may be more widespread than heretofore has been observed.

The similarity of conjugation in *Trichodina pediculus* to that of certain members of the Vorticellidae was first pointed out by Busch (1855). Stages in conjugation among certain of the Urceolariidae have since been recorded and the same comparison made. Caullery & Mesnil (1915) reported stages in conjugation in *Trichodina patellae* (Cuenot) from a species of fresh-water mollusc. Peshkowsky (1923) described conjugation in *T. steinii* and commented that this process was similar in all essential features to that observed in the Vorticellidae. Anisogamous conjugation was described by Zich (1928) for *Urceolaria korschelti*, and by Hunter (1936) for two types of *Trichodina* found in the intestine of sea-cucumbers. Diller (1928) described in detail endomixis in *Trichodina* from tadpoles. He suggested that some of the evidence of conjugation presented by Caullery & Mesnil, Peshkowsky and others were most likely stages in endomixis. The endomictic stages described by Diller, however, conform closely to the nuclear reorganization in the post-conjugative stages described for *Trichodina spheroidesi*. Diller makes a distinction between endomictic individuals and conjugating forms on the basis of differences in shape of macronuclear fragments. Since the fragments in Diller's material are not unlike those observed in some of the stages in conjugation in *T. spheroidesi* this distinction cannot be supported. Any other differences that Diller may have noted are, in all probability, the result of examining too few samples of conjugation which he stated was present in his material. That he may have misinterpreted post-conjugation for endomixis is supported further by the similarity of his figure (Pl. II, Fig. 14) of the first micronuclear division to the figure of the zygote nucleus in *Vorticella nebulifera* shown by Maupas (1888) (see Doflein, Fig. 310) and in *Trichodina spheroidesi* (Pl. II, Fig. 13).

SUMMARY.

1. *Trichodina spheroidesi* and *T. halli* spp. nov. from the gills and skin of puffers (*Spheroides maculatus*) and other marine fishes from the New Jersey and the New York coast are described.

2. The processes of fission and conjugation are described for *T. spheroidesi*.

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EXPLANATION OF THE PLATES.

Anisogamous conjugation in *Trichodina spheroidesi*. All figures drawn from material stained with iron-hematoxylin. $\times 950$.

PLATE I.

- Fig. 1. Start of conjugation.
 Fig. 2. Macronucleus in the process of fragmentation. Note lumping of nuclear material. Micronucleus in pre-metaphase stages of meiosis.
 Fig. 3. First macronuclear fragmentation. Micronucleus still in pre-metaphase stage.
 Fig. 4. Further macronuclear fragmentation.
 Fig. 5. Metaphase of meiotic nucleus clearly evident. Fragmentation of the macronucleus continues.
 Figs. 6, 7, 8. Continued meiotic division and completion of fragmentation of the macronucleus into many spherical and oval shaped bodies of various size.

PLATE II.

- Fig. 9. Continuance of the meiotic process seen in Fig. 8.
 Fig. 10. Second micronuclear division. Note persistent gametic micronucleus and the degeneration of other three micronuclei in each conjugant.
 Fig. 11. Gametic nuclei in an early stage of fusion to give rise to the synkaryon. The other micronuclei completely disappeared. Note the completion of cyto-

plasmic continuity between the conjugants.

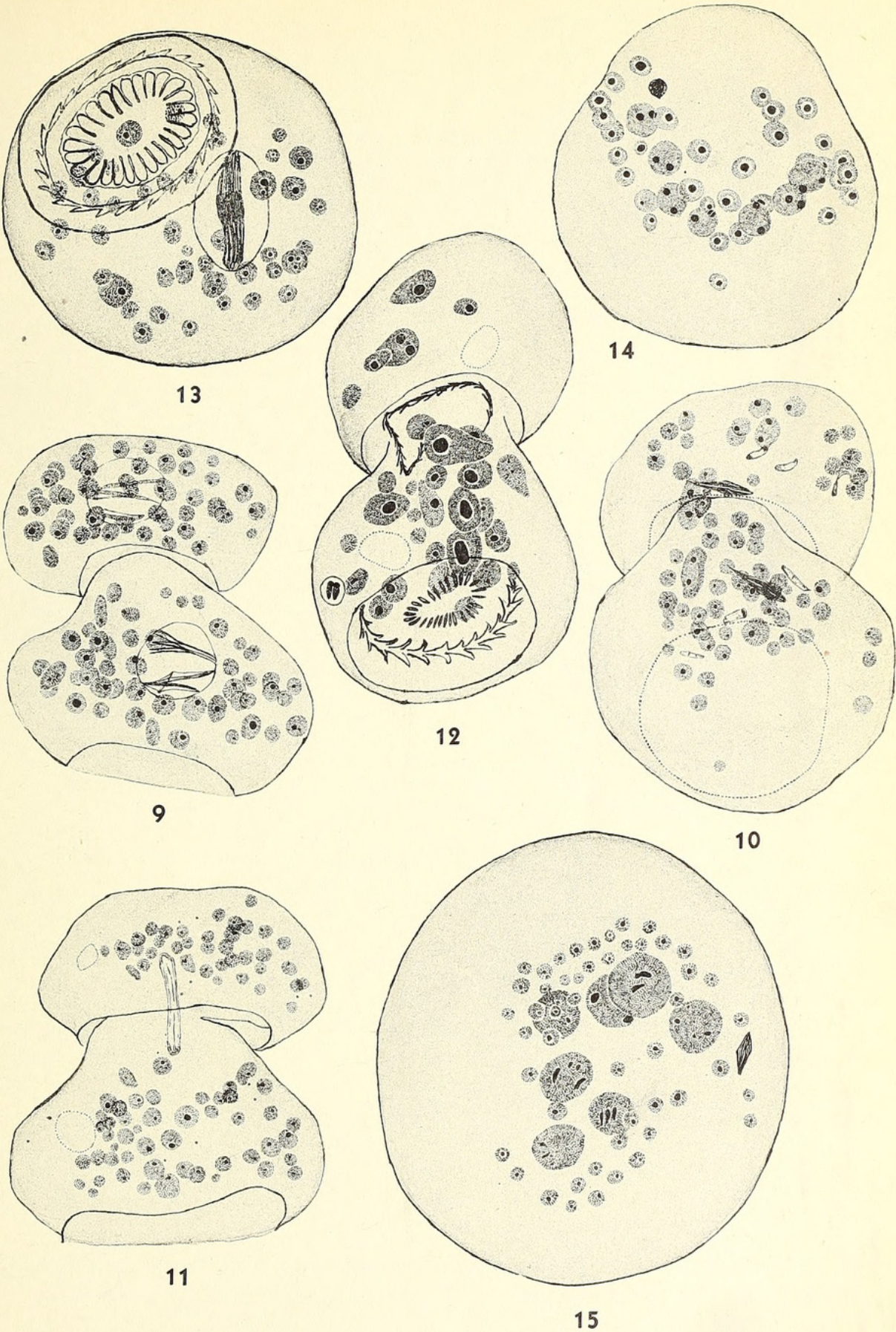
- Fig. 12. The cytoplasmic contents of the microconjugant pass into the macroconjugant. The new denticulate ring develops while the old ring is resorbed. In this individual the macronuclear fragments are coarser than in the preceding stage.
 Fig. 13. Post-conjugant stage. Macroconjugant with large zygotic nucleus in metaphase stage. New denticulate ring and remnant of the old ring still present.
 Fig. 14. Initial stage in development of the macronuclear anlage. There are seven of these larger bodies present, indicating that three divisions of the zygotic nucleus had taken place.
 Fig. 15. Further development of the macronuclear anlage. The start of the first binary fission. Micronucleus in metaphase.

PLATE III.

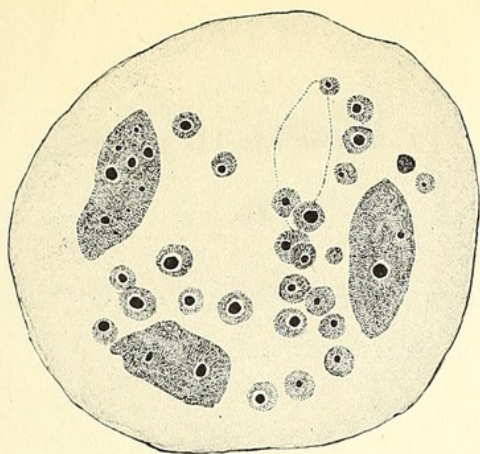
- Figs. 16, 17. Daughter cells showing the three and four macronuclear anlage distribution.
 Figs. 18, 19. A two and five distribution of macronuclear material.
 Fig. 20. Each cell will continue to divide until only a single macronuclear anlage is present in each individual. The last step in this process is shown in this figure.



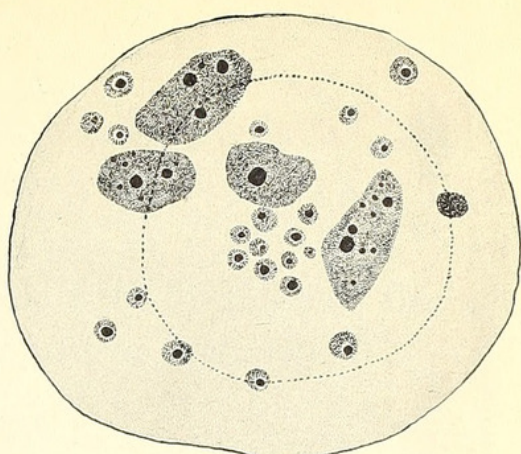
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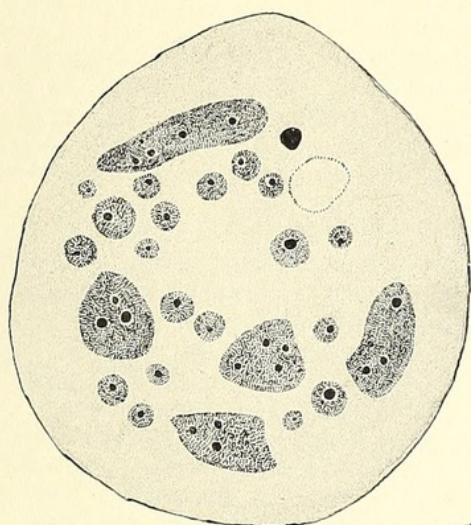
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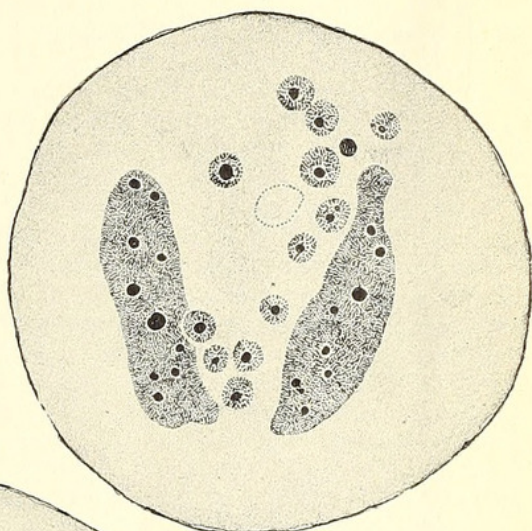
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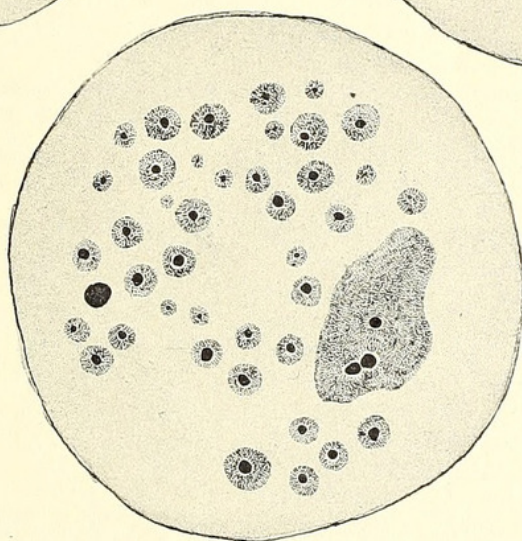
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