

~~4653~~ 145350 (306)

ALLAN HANCOCK FOUNDATION PUBLICATIONS
OCCASIONAL PAPER NUMBER 5

TRICHOCHONA LECYTHOIDES,
A New Genus and Species of Marine Chonotrichous
Ciliate from California, with a Consideration of the
Composition of the Order Chonotricha Wallengren, 1895

(PLATE 1)

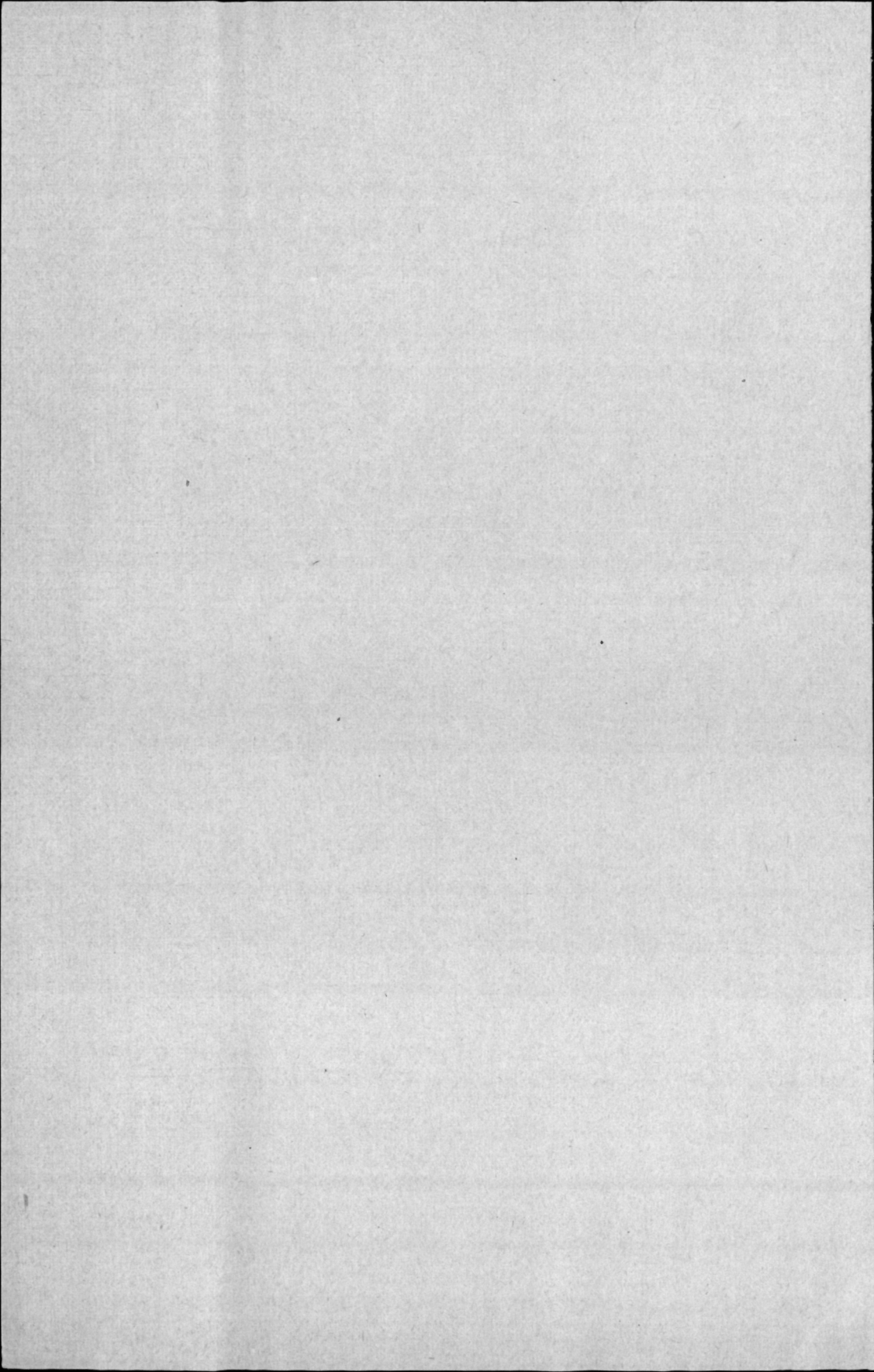
BY
JOHN LUTHER MOHR

Guido PERSOONE
ASSISTENT
Lab. voor OEKOLOGIE
Ledeganckstraat, 35
GENT



THE UNIVERSITY OF SOUTHERN CALIFORNIA PRESS
LOS ANGELES, CALIFORNIA

1948



To Jovan Hadži
with best regards
John Mohr

TRICHOCHONA LECYTHOIDES,

A New Genus and Species of Marine Chonotrichous
Ciliate from California, with a Consideration of the
Composition of the Order Chonotricha Wallengren, 1895

(PLATE 1)

By JOHN LUTHER MOHR

ALLAN HANCOCK FOUNDATION PUBLICATIONS
OF
THE UNIVERSITY OF SOUTHERN CALIFORNIA
OCCASIONAL PAPER NUMBER 5
ISSUED AUGUST 25, 1948
PRICE \$1.00

THE UNIVERSITY OF SOUTHERN CALIFORNIA PRESS
LOS ANGELES, CALIFORNIA

TRICHOCHONA LECYTHOIDES,

A New Genus and Species of Marine Chonotrichous Ciliate from
California, with a Consideration of the Composition of the
Order *Chonotricha* Wallengren, 1895.

(PLATE 1)

BY JOHN LUTHER MOHR

Although *Spirochona gemmipara* Stein 1852, first-described of the chonotrichous ciliates, has been reported from the gill-leaves of fresh-water *Gammarus pulex* from England to Lake Baikal, relatively few members of this order have been described since 1852. All of these, excepting species of *Spirochona* itself, have been found as ectozoans on marine or brackish water crustaceans of Europe. With the exception of Swarczewsky's (1928a) study of the nuclei of *S. elegans*, there has been no cytological work on any of them in half a century.

An infestation of a chonotrich discovered on a marine amphipod, *Amp(h)ithoë* sp., from the hold-fast of a kelp taken at Redondo Beach, Los Angeles County, California, in October, 1945, provided material for the present study. About 2,000 ciliate individuals were counted chiefly on the feather-like pleopods or swimmerets, but also on the pereopods of the host. A *Cothurnia* (Ciliata, Peritrichida) was present in smaller numbers at the bases of the same appendages.

The chonotrichs were examined in life very briefly as previously described species were reported to be excessively fragile; subsequent study of the fixed and mounted individuals confirmed the reports. The pleopods were dissected from the host with iridectomy scissors and dropped into Bouin's, Hollande's or Heidenhain's "Susa" fluid. Clusters of the protozoans fixed in Bouin's fluid were stained with Lynch's precipitated borax-carminc (Carminc Red Ia, Dr. G. Grübler, Leipzig) or Heidenhain's hematoxylin. As the carminc stain gave poor definition of essential structures, some of this group were re-stained with Galigher's standard alum hematoxylin which, under these conditions, gave unsightly but more useful preparations. Clusters of Hollande- and "Susa"-fixed animals were impregnated with protargol (Bodian, 1937): these developed (in the same sense as in photography) the pattern of basal granules of cilia which sets off the Order Chonotrichida from other ciliate groups. As fixative for this impregnation, "Susa" was superior to Hollande's fluid.

Heidenhain's hematoxylin after "Susa" or Hollande's gave the most generally useful results, although the basal granules were demonstrated less obviously than by the Bodian impregnation.

Professor Harold Kirby of the University of California, Berkeley, kindly lent a series of commercially prepared slides of *Spirochona* sp. on the gill-leaves of an unidentified *Gammarus*. On being questioned, the suppliers stated that the slides were prepared in England. These preparations made possible a number of comparisons. Mr. K. Stephensen, Zoologiske Museum, Copenhagen, identified the host amphipod. Dr. Albert H. Travis, Department of Classical Languages of this University, advised the writer of the proper form for the name of the ciliate. Mr. Anker Petersen, staff artist of the Allan Hancock Foundation, prepared the figures. To all these and to the Director of the Foundation the writer acknowledges his indebtedness.

TRICHOCHONA, new genus

Diagnosis.—Large, elongate chonotrichs with long stalk; pellicle heavy; collar-like ectoplasmic funnel leading to peristome is simple, single; ciliary patches, two (one with rows parallel to funnel rim [horizontal field], the other with diagonal rows [adoral field] leading to cytostome at deeper end) occupying three quadrants of the funnel, the remaining quadrant being unciliated; budding frequent, buds rising on side opposite cytopharynx in deep pouch (marsupium) which persists in mature individuals; single macronucleus, one to four micronuclei; ectocommensal on marine crustaceans.

Trichochona differs from the older genera as follows: from *Heliochona* in being stalked and in lacking supporting radiating striae or spines in the wall of the peristomal funnel; from *Kentrochona* in having a longer stalk and a slender body, and in lacking thorn-like structures in the funnel wall; from *Stylochona coronata* in having a single (as opposed to a concentrically double) funnel and in lacking radiating striae in the funnel wall. *Chilodochona*, in contrast, lacks a peristomal funnel altogether and *Spirochona*, the sole fresh-water genus, has a funnel wall folded into a helicoid spiral.

The genus is named *Trichochona* (θρίξ, τριχός, hair, + χώνη, funnel) in reference to the thick patches of cilia in the peristomal funnel.

Type species.—*Trichochona lecythoides*.

Trichochona lecythoides, new species

Diagnosis.—Peristomal funnel with horizontal ciliary lines up to 32, diagonal lines, about 20; cilia not completely encircling funnel; pellicle, thick; resting macronucleus oval to long ovoid; no contractile vacuole. Total length, 81-164 μ ; body length, 35-86 μ ; maximum body width, 3-28 μ (latter dimension in budding individuals); funnel length, 8-21.5 μ ; stalk length, 16-51 μ ; macronuclear length, 9-22 μ . Epibiotic on lateral branches of pleopods (and other parts) of *Amp(h)ithoë*.

Type host.—*Amp(h)ithoë* sp. (Gammaridea, Amp(h)ithoëidae), female.

Type locality.—Redondo Beach, Los Angeles County, California.

Co-syntypes.—U.S.N.M. No. 22728 and AHF no. 1000 on parts of pleopods of a female *Amp(h)ithoë* taken in October, 1945.

Para-syntypes.—Remainder of type infestation.

General morphology.—A comparative study of *Trichochona lecythoides* is difficult because previous studies of marine chonotrichs antedate modern cytological methods. In general chonotrichs may be described as having three regions: a prestomal funnel or collar, a body and an attachment device. In mature *T. lecythoides*, the funnel is separated from the body by a deep constriction (neck). The funnel is characteristically shaped like the top half of an hour glass and is tilted a few degrees from the long axis of the body. The highest part of the funnel rim is on the side of the marsupium and of the adoral ciliary field (discussed below). Within the funnel are two patches of cilia, the adoral and horizontal fields. If one focuses on the basal granules of the adoral field in an example in which it may be viewed in profile (particularly in Bodian preparations), the field is seen to extend from a point just below the rim downward to the level of the cytostome. If, on the other hand, the adoral field be examined in a protozoan in which it may be seen in full view and at high focus, it may be seen to be composed of diagonal lines of granules (the diagonals may be traced from top to bottom from either side) and to be broader at the rim than near the cytostome. The horizontal field, in this case, is contiguous at the left, composed of 24 to 32 rows paralleling the rim of the funnel. To the right of the adoral field there is a cilia-free area extending about one quadrant of the funnel. The cilia are well-defined, about 3 to 4 μ in length, beating within the lumen. The funnel in young individuals is set off from the body by a shallower neck constriction and the sides are more nearly parallel with those of the body. The number of ciliary lines in both adoral and horizontal patches is smaller than with adults.

The cytosome is of slender, vase shape, tapering posteriad to the junc-

tion with the stalk. It is somewhat asymmetrical, extending farther forward at the side of the adoral field of the funnel. It is bounded by a pellicle of definite thickness (ca. 0.5μ), the outer surface having a very finely pebbled appearance. The pellicle is stained faintly with iron hematoxylin (when differentiation is satisfactory for nucleus and fibrils), except near the stalk where it destains completely. It would appear that the living pellicle exerts pressure upon the cytoplasm because a number of moribund individuals observed show the cytoplasm bulging from the funnel, the cytostome protruding beyond the funnel rim. This would seem to indicate that the thinner cytoplasmic membrane of the funnel lining weakens and that the protoplast is extruded at this region because of the elasticity of the stout pellicle. It is notable, also, that the pellicle and stalk persist for a time after the chonotrich dies and the protoplast disintegrates.

At the level of the macronucleus of mature individuals, the pellicle is perforated on the side corresponding to the adoral field; here a thinner-walled sac opens. This sac, the marsupium, runs posteriad along the side. In it buds are formed singly (as described below), the structure persisting throughout adult life. It has not been observed in juvenile forms (Plate 1, fig. C). The marsupium of *Trichochona* has its counterpart in a shallow sac or groove in *Kentrochona*, but the relatively complete sheltering of the embryo seems to be confined to this genus. The marsupium contributes to the asymmetry of the animal.

The nuclear complement of *Trichochona lecythoides* is somewhat variable. Minimally there are one macronucleus with a darker (chromophile) and a lighter (chromophobe) zone and a single micronucleus. The macronucleus is prominent, oval or elongate, lying in the anterior third of the cytosome. It usually lies at an angle to the long axis of the cell, but not crosswise. In simplest form the darker portion lies anterior, is coarsely spongelike with spherical, lighter bodies in the interstices, and occupies about half the nucleus; the lighter portion is finely granular with one to about six spheroidal, homogeneous darker bodies lying in clear vesicles.

There are a number of common departures from this simple condition. The chromophobe zone may lie anterior. There may be two or even three bands of either sort alternating from anterior to posterior. Frequently that part of the chromophile zone bordering the chromophobe area is homogeneously and intensely black rather than spongelike. Commonly, when there is a single dark body in the chromophobe zone, it is central and prominent. It is apparently homologous to the "micronucleus" of *Spirochona spp.* as Swarczewsky (1928a) interprets the structure. At the pos-

terior end of the macronucleus, included within the membrane, often lies a homogeneous, lightly staining, spherical body. This is of uncertain significance.

Usually examples of another sort of structure may be seen in the cytoplasm posterior to the macronucleus. These are cytoplasmic inclusions in Swarczewsky's opinion, but like Balbiani (1895-on *Spirochona*), Hertwig (1877-on *Spirochona*), and Doflein (1897a-on *Kentrochona*), the writer considers them true micronuclei. They are about $2.5\ \mu$ in diameter and in iron hematoxylin, preparations show a delicate but well defined membrane and small, siderophile granules. Spindles (the membrane persists during mitosis) attain a length of $4\ \mu$ or more at metaphase(?). In protargol preparations micronuclei can be detected only occasionally and dimly. The writer's Feulgen preparations have been unsatisfactory. The number of micronuclei is not constant and may not be characteristic, although mature individuals most often have two or three (uncommonly four) and immature forms usually have one.

With the ciliary fields the fibrillar system is, in the writer's opinion, of such characteristic form that it provides a common variable of primary importance within the order Chonotrichida and a fundamental structural difference from the Peritrichida with which the funnel animalcules have been placed mistakenly. The system is much less complex than that of the peritrichs having only (1) the parallel fibrils integrating the basal granules in the adoral and horizontal ciliary fields, (2) a group of longitudinal fibrils bounding the cytopharynx, and (3) short connectives in the cytostomal region linking the ciliary fields to the cytopharyngeal fibrils. The cytostome itself is just ahead of the neck and on or near the longitudinal axis. It appears to lie open, well demarked by the longitudinal fibrils which extend backwards, being deflected by the macronucleus always to the side opposite the marsupium. In healthy individuals the fibrils may not all be counted, but one may detect at least three. In individuals with extruded nuclei and cytopharynx pulled forward, the fibrils are spread out and as many as six fine lines can be seen. These fibrils continue below the macronucleus disappearing near or a little below the middle of the cytosome.

The cytoplasm in general is finely granular (coarser with Hollande-Bodian technic) with no differentiation in the body region between ectoplasm and endoplasm. There is no contractile vacuole and food vacuoles lack precise membranes, appearing as clearer areas within the protoplast reflecting the shape and degree of disintegration of the food particles. The food is a filamentous rhodophycean which may often be seen in undigested and recognizable condition within the vacuoles.

The mechanism of attachment of body and stalk is of very considerable interest in itself and as a structure setting the chonotrichs off from the peritrichs. The pellicle, which is about 0.4 to $0.5\ \mu$ in thickness over the rest of the body approaches $1.0\ \mu$ just above the stalk, then thins as it turns inward almost at a right angle (as may be observed particularly well in partially decomposed examples). The cytosomal pellicle is continuous with the outer layer of the stalk which begins with a heavy annulus, deeply staining or semi-transparent, against which the end of the body fits closely. Its diameter is about $5\ \mu$ in mature individuals.

Extending forward from the annulus into the cytoplasm of the body is an irregular tuberous structure bounded by a fine membrane and containing a clear cytoplasm. The structure extends 7 to $21\ \mu$ into the body and is 2 to $3.5\ \mu$ in diameter. At the posterior end there appear faintly alternate lighter and darker longitudinal lines (about 10 of each) on the membrane. These extend into the stalk where they become very distinct forming the middle layer. The clear cytoplasm is continuous with the core of the stalk. In life there is thus a continuity of the pellicle with the outer layer of the stalk providing for an elastic hinge, while the right angle form of the base of the cytosome and the adjacent stout annulus of the stalk provide a check to excessive movement. The tuberous body, which can be displaced to the side as the animal moves, by its continuity of membrane with the striped middle layer (about $2.25\ \mu$ across the cylinder) of the stalk and by continuity of cytoplasm with the clear core (about $1.25\ \mu$ in diameter) of the stalk apparently form a sort of elastic-bounded hydraulic stabilizer. The whole constitutes an admirably functional universal joint operating within a useful conical area of perhaps 90° . Junction with the "barb" of the pleopod is effected by an irregular expansion of the base of the stalk into a haptere extending about 10 to $14\ \mu$ along the "barb" (transverse measurement not made). A similar cementing material and a modification of this holdfast mechanism has been reported for the other chonotrichs of which precise descriptions have been published.

The specific name of *Trichochona lecythoides* is taken from *λεγκυθος*, a tall, cylindrical jar with flared mouth, and *ειδος*, form.

Budding.—A recurrent and remarkable phenomenon of the Chonotrichida is budding (gemination, gemmazione, Knospung), which has been reported for most species and which reaches an extreme of reduplication in Doflein's (1897b) "*Kentrochonopsis multipara*." Examples of budding are common in the *Trichochona* material and are present in the *Spirochona* slides lent by Professor Kirby. In all cases the process would

seem to be a modified binary fission rather than a wholly different mechanism. The parent individual retains its identity and there are no important changes in the structures of funnel, cytosome or stalk of *Trichochona* bearing fully formed buds. Except for the presence of a deep, lateral bud-pouch or marsupium, which appears to be much more highly developed than related structures in other chonotrichous genera (*Kentrochona* has a small pouch), budding in *Trichochona* may be considered characteristic of the order.

First indication of budding in *Trichochona* is a slight bulging of the cytosomal cytoplasm into the most posterior part of the marsupium. This is noticeable because it stretches slightly the outer wall of the pouch and makes a gap between the cytoplasm just anterior to the bud and the marsupial outer wall. At this time mitosis of the micronucleus can be seen in favorable material. Stages slightly more advanced show larger buds bulging laterally and now also forward toward the mouth of the marsupium. At this stage what appears to be a vesicle forms a little anterior to the midpoint of the bud. The nucleus of this stage (represented by a very few examples) has not been observed. Immediately after this faint lines may be seen in the vesicle and about this time a macronucleus is always discernible in the bud. In *Spirochona* the bud macronucleus is stated to have been produced by division of that of the parent individual; in *Trichochona* the macronucleus of the parent has not been demonstrated to enter into the budding process. However, too few animals in the critical stage of division have been collected to permit conclusions. After the stages in which faint lines may be seen in the vesicle, these lines become plainly visible and elongate along the long axis of the bud. Anteriorly they are seen as series of granules; posteriorly, as a meshwork of fine fibrils. At this time one may still find continuity between some of the lines of granules and some of the posterior fibrils. These relationships are demonstrated strikingly in both Heidenhain's hematoxylin and Bodian's protargol preparations. The lines of granules (basal granules of cilia) now become arrayed in two unequal groups not unlike the fingers and closely apposed thumb of a mitten. It is assumed because of the relative sizes of the two groups that these are respectively the horizontal and adoral ciliary fields, but it has not been possible to find intermediate stages to establish this as fact. The mesh of fibrils at the posterior end of the bud curves toward the parent. In advanced buds (larvae), it is no longer possible to trace connection between the anterior and posterior areas. The latter becomes a compact stalk, but in the writer's material one cannot determine how the three layers of the stalk are differentiated embryonically. It has been seen,

however, that the peristomal ciliary mechanism and the fibrillar portion of the stalk develop from a common anlage and that this rises *de novo* in the cytoplasm of the embryo.

In buds which are so large as to protrude from the marsupium, the ciliary fields lie on the walls ("lips" of Wallengren, 1895) of either side of a shallow cleft, but there is still no funnel formed. These are the most advanced bud stages in the infestation studied. Youngest solitary individuals observed have a small funnel with sides nearly parallel to the sides of the cytosome rather than flared. During the process of growth from the stage in which granules first may be seen to lie in two fields, to the mature *Trichochona*, the number of rows in the ciliary fields increases. The least counted in the horizontal field of a bud is about six (the writer has been uncertain where lines left off in the curved surface); in a young solitary example, the number was 12 while in individuals with a marsupium the number has ranged from 18 to 32. (In all these counts the writer has tried to be positive, but in the counting of a series of like dots at the limits of resolution and at changing foci, he has been able to give only mean counts for fields).

Relations with host.—Ecologically, the Chonotrichida occupy a highly specialized niche. So far as known they are all commensal on active crustaceans and on these they locate upon structures which receive a maximum amount of dissolved oxygen. Swarczewsky's (1928a) statement about his Lake Baikal species, *Spirochona elegans*, seems adaptable to all reported members of the order: "Wenn wir alle obenerwähnten Beobachtungen zusammenfassen, so kommen wir zu dem Schlusse, dass unsere *Spirochona* eine sehr zarte Form ist, die sehr stark auf die Wassererneuerung angewiesen ist. Sie nimmt Platz: 1. auf solchen Stellen des Kiemenblattes, wo wir den stärksten Wasseraustausch annehmen müssen, 2. auf solchen Kiemenblättern, die in kräftigere Bewegung bebracht werden und 3. auf Wirtsindividuen, die zu starken und schnellen Bewegungen fähig sind." Swarczewsky's first and second points need little modification to describe the situation in *Trichochona lecythoides*: the ciliates fasten themselves on the pleopods or swimmerets, which by rapid beating effect a rapid water exchange, and on those pleopods, well out on the "barbs" where the arc described is greatest. In a sense his third point applies to all of the ectozoans (Holotrichida, Spirotrichida, Peritrichida, Suctoria) of the edriophthalmous crustaceans, a factor which relates at least in part to the longer intermoult of mature individuals. In the case of *T. lecythoides*, it has the survival value which Swarczewsky implies.

In great abundance on the "barbs" and "barbules" of the appendages

and on the stalks of the *Trichochona* are large and small filaments of a rhodophycean, probably *Bangia*. Some members of this algal genus are restricted to the high tide line on rocks and so may be presumed to be macroaerophilic like the *Trichochona*. They appear to constitute the principal and almost the only food of the ciliates in this infestation. The sole exception observed was a diatom frustule in the cytoplasm of one individual.

From the pleopods the *Trichochona* hang funnel-down in relation to the amphipod and anteriorly or posteriorly as regards the long axis (Plate 1, fig. A) because the hapteres have sufficient room for attachment only on the anterior and posterior surfaces of the "barbs," the closely spaced "barbules" leaving too little room at the sides. In addition to the individuals on the pleopods (which comprise the greater part of the infestation), there are some on the posterior walking legs (pereiopods) and a few that have escaped observation may be on other parts of the host. Those on the pereiopods are distally placed. In contrast, the *Cothurnia* present are located at the bases of the same appendages.

Although the pleopods of the amphipod were seemingly heavily freighted with ciliate commensals, most of the 2,000 odd individuals being here, it cannot be stated that the movement of these appendages was noticeably retarded. Indeed, observations of the living protozoans were made difficult and unsatisfactory by the continual and rapid pleopodal beat.

Host specificity.—Although Wallengren (1895) found a case of what appeared to be straying of *Chilodochona* from the normal host to a hermit crab in a marine aquarium containing both, there is as yet no reported case of a chonotrich commensal on anything but a crustacean. *Trichochona lecythoides* is known from a single host individual and so contributes little to a concept of the group except that it occurs upon an amphithoid, a member of a family not hitherto reported to harbor chonotrichs, but within the order from which most known funnel animalcules have been taken. It may be noted that Swarczewsky (1928b) has found *Spirochona elegans*, the most common Baikal species, on 18 species of gammarid amphipods scattered through ten genera, but never upon any other host animals. The relationship would seem to depend in the main upon the ecological conditions prevailing about the affected parts of the crustaceans.

Composition of the order **Chonotrichida**.—Probably because the funnel animalcules were known first through *Spirochona gemmipara* Stein 1852 and because this infusorian was readily accessible to cytologists (Balbiani, Bütschli, R. Hertwig and Hickson, among others, worked on

it) throughout Europe, errors in the interpretation of the fundamental peristomal structure were made and propagated. Had equal attention been given *Chilodochona*, *Trichochona* or even *Heliochona*, genera with simple or little modified funnel architecture, such errors might have been avoided. However, the funnel of fully developed *Spirochona* (but not that of young *Spirochona*) a helicoid spiral, resembles superficially the spiral peristome of vorticellids. This, coupled with the vase-like, unciliated body, caused Stein (1854) to place his genus *Spirochona* (with *Dinophysis* Ehrenberg 1839) in a new family, Spirochonina, alongside the vorticellines. Subsequently he (1859) erected the order Peritricha to contain them. It became customary (*v. e.g.*, Delage & Hérouard, 1896) to divide the peritrichs into the right-wound forms (dexiotrichs, from δεξιός, right) like *Vorticella* and the left-wound forms (scaiotrichs, from σκαίος, left), *Licnophora* and the spirochonines. That such a classification did not express phyletic relationships, was suspected early. Claparède and Lachmann (1858) placed *Spirochona* in an appendix to the vorticellines noting that affinities were uncertain. Eismond (1890, 1895), who appears to be the first to study the peristomal structure critically, demonstrated that earlier workers (and more recent investigators made the same mistake) were not dealing with homologous structures in the vorticelline peristome and the spirochonine spiral funnel, and that the peristomal structures of *Spirochona* were, in fact, curved in the opposite direction from the spiral of the funnel.¹

Beginning in 1891, Wallengren made the series of studies of ciliates of Swedish waters which form the basis of any proper study of the group today. From a thorough study of *Licnophora auerbachii* (a commensal of the nudibranch, *Doris muricata*), published in 1894, he was able to show that the genus has only superficial resemblance to the Peritrichida and that its relationships must be sought among other ciliate groups.²

The following year Wallengren elucidated the structure of the remaining "scaiotrichs" establishing for them the "Sektion" (= Order) Chonotricha (from χώνη, funnel, + θρίξ, τρίχος, hair). Among the characteristics which Wallengren used to separate the chonotrichs from the

¹ It remained for Swarczewsky (1928b) to find that there occur on Lake Baikal gammarids species of *Spirochona* which have counterclockwise spirals (*Sp. gemmipara*, *Sp. pusilla*), clockwise spirals (*Sp. patella*) or even both within a single species (*Sp. simplex*, *Sp. globulus*). It would be most useful to have detailed accounts of the cytostomes of these several species.

² For an authoritative review of this separate problem, *v.* Balamuth, Wm., J. Morph., vol. 68, pp. 241-269, March, 1941.

peritrichs are the ciliation of the peristome, the lack of sphincter action of the peristome or cytostome, lack of or slight contractility of the cytosome and non-contractility of the stalk or haptere of the chonotrichs. He pictures (1895, fig. 2) the horizontal and adoral ciliary fields (of *Heliochona*) as continuous lines, probably basing his sketch on the movement of the cilia in life rather than upon study of the lines of granules themselves. He did, however, show them in their proper morphological and functional relationship.

Wallengren further divided the order into the families Chilodochonina (new, with two [?] species having undeveloped funnels, both found as commensals on the mouth-parts of crabs) and Spirochonina (Stein, 1854) which he emended to contain *Spirochona* Stein 1851, *Stylochona* Kent 1881, *Heliochona* Plate 1888 and *Kentrochona* Rompel 1894, all with well developed funnels.

Although Wallengren's separation of the chonotrichs from the peritrichs is unassailable and although he published his account of the new order in German (1896) as well as in Swedish, zoölogists, with notable exceptions (Enriques, 1908; Poche, 1913), overlooked or disregarded this point of view. Thus Hickson (1903), Hamburger and von Buddenbrock (1911), and even Kühn (1932) retain these forms in the Peritrichida. Penard (1922), apparently ignorant of Wallengren's contributions, recognized the incompatibility of the union and raised Scaïotricha (*sine Licnophora*) to ordinal rank. The authority of Reichenow (1929) and Kahl (1933, 1935) now assures the acceptance of Wallengren's classification and his name (now usually modified to Chonotrichida [nec Conotrichida, Pearse, 1942] following the usage of *Zoological Names*) for the order.

Within the order there now appear to be three reasonably well defined groups of species. The writer considers it likely that marine chonotrichs evolved before freshwater forms of the order; this thesis is supported by the distribution of the groups and by the greater number of representatives and variability of structure of the marine genera. It is considered self-evident that a funnel-less form must have preceded those chonotrichs with well-developed funnels (although in the consideration of any modern species, absence of such an apparatus might be explained as simplification) and that simple, unadorned funnels must precede the variously modified ones. The embryology of *Trichochona* as seen in the present study and that of *Spirochona* as described in the literature (Hertwig, 1877; Swarczewsky, 1928a) support this point of view. A chonotrich embryo develops

cilia within a simple groove bordered by "lips" such as are present in adult *Chilodochona*. One may speak then of a chilodochonoid larva or embryo. Subsequently, a collar-like extension of the "lips" forms the simple funnel of both young *Trichochona* and young *Spirochona*. (One prefers not to call this trichochonoid, for although *Trichochona* is considered more primitive than the other genera now known, it cannot be considered basic to the funnel-bearing series.) Logically one must also assume that stalkless species must have existed before stalked ones; *Trichochona* has a well-developed stalk. Further, *Trichochona* has a deep marsupium which is clearly a derived rather than a primitive character.

Granted these premises, the writer divides the Order Chonotrichida into three families:

Chilodochonidae Poche, 1913 (from Chilodochonina Wallengren, 1895), unigeneric, for *Chilodochona* with two (?) species ectocommensal on the mouth-parts of marine brachyurans, funnel-less.

Stylochonidae, *fam. nov.*, with *Stylochona* Kent as type genus, *Heliochona* Plate, *Kentrochona* Rompel (+ *Kentrochonopsis* Doflein) and *Trichochona*, *gen. nov.*; all ectocommensals of marine crustaceans (*Nebalia*, amphipods, isopods), with or without stalk, all with well-developed funnels, but in no case with funnel thrown into spiral folds.

Spirochonidae³ Grobben 1904 (emend. from Spirochonina Stein 1854), here restricted to the genus *Spirochona* Stein 1852, ectocommensals of fresh-water gammarids; funnel thrown into spiral folds, stalkless. *Spirochona* (= *Heliochona*) *scheutenii* Stein 1854 is a stylochonid. "*Spirochona crystallina*" Canu 1888 is *nomen nudum*; since no spirochonid, *s. s.*, is known to occur in salt water, it will be a point of interest to investigate the chonotrichs of *Limnoria lignorum* from which this undescribed form was reported.

On the basis of the structure of known forms and of their embryology, fragmentary as the knowledge is, it is possible to deduce something of probable lines of evolution in the order. The most primitive recognizable chonotrich would be an unstalked ciliate somewhat similar to *Chilodochona* with an oral groove rather than a funnel. From this hypothetical form, *Chilodochona* may be derived by the development of a stalk. The stylochonid stock would presumably spring from the same hypothetical form by the development of lips about an oral groove into a complete funnel. The most primitive member would be very like a *Trichochona*

³ Poche (1913) gives the year as 1904, but 1905 is the earliest edition of Grobben's text which has been available to the writer.

without either stalk or marsupium or like a *Heliochona* without rays in the funnel wall. It may be anticipated that some such form will be found as the group is studied further. From such a funnel animalcule, *Trichochona* would arise through the development of stalk and marsupium and *Heliochona* by the elaboration of funnel-rays. *Stylochona coronata*, with an outer rayed funnel and an inner plain funnel may be considered a derivative of the *Heliochona* stem while *Kentrochona* might have been derived from the *Trichochona* stem by elaboration of the funnel thorns or from the *Heliochona-Stylochona coronata* series by growth of a stalk and modification of rays.

The spirochonids would be most easily derivable from early stylochonids, unstalked forms with unornamented funnels, by extension of the funnel ectoplasm into whorls of varying complexity. The embryology of *Spirochona* strongly suggests this course of evolution. The spirochonids constitute an extremely compact group of presumably recent origin. So far the genus *Spirochona* has been taken only in Palearctic inland waters; if species are not subsequently discovered beyond the limits of this region, it will be further presumptive evidence of the youth of the group.

Geographical distribution.—The finding of *Trichochona* in Pacific waters shows that the chonotrichs are not confined to Palearctic waters whence all previous records come. *Chilodochona* has been taken only in Scandinavian waters. *Trichochona* is known from the southern Californian littoral. *Heliochona* was described from Dutch waters near Amsterdam (Stein, 1854) and was found subsequently in Wismar Bucht (Rentsch, 1860) and the North Sea (Plate, 1888). *Kentrochona* has been reported from Trieste and Rovigno in the northern Adriatic (Rompel, 1894; Doflein, 1896a and b). *Stylochona, sensu latu*, is reported only from Jersey in the English channel (Kent, 1881).

Spirochona (Stein, 1852) is the oldest and best known genus of the order. *S. gemmipara*, the type species, was taken originally at Tharand, near Dresden, Germany. Subsequently it has been taken in France (Canu, 1886), Belgium (deGeest, 1933), Switzerland (Roux, 1900, 1901; Sakowsky-Campioni, 1906; André, 1912; Pénard, 1922), at Freiburg (Henderson, 1905), Munich (Plate, 1886) and near Berlin (Claparède and Lachmann, 1858) in Germany, in Poland (Eismond, 1895) and in Siberia (Swarzewsky, 1928b). As to the identity of *Spirochona* in England, there is some doubt. Hickson (1903) pointed out that while Hertwig (1877) considered three to be the normal number of micronuclei for *Spirochona gemmipara*, those examined at Manchester had only one.

Professor Harold Kirby lent the writer slides of a *Spirochona* said to have been prepared at Manchester. Individuals are too few to allow any conclusions, but in ten consecutively examined individuals, five had two micronuclei, two had three, one had four and the remaining two were indistinct. Considering the value of micronuclear number as a specific character in the Ciliata, it is conjectured that this may be a separate species or race from that which Hickson observed. Likewise a critical re-examination of "*Spirochona gemmipara*" throughout its range is indicated.

The remaining species of *Spirochona*, eight in number, were described by Swarczewsky from Lake Baikal gammarids and reflect in a small way the extraordinary endemic development of gammarids in that lake.

LIST OF REFERENCES

ANDRÉ, ÉMILE

1912. Infusoires. Fascicule 6 in Catalogue des Invertébrés de la Suisse. Genève, Georg & C^{ie}.

BALBIANI, ÉDOUARD GÉRARD

1895. Sur la structure et la division de noyau chez le *Spirochona gemmipara*. Annales de Micrographie, vol. 7, pp. 241-260, 289-312, plate II.

BODIAN, DAVID

1937. The staining of paraffin sections of nervous tissue with activated protargol. The role of fixatives. Anatomical Record, vol. 69, pp. 153-162.

BÜTSCHLI, OTTO

1877. Ueber den *Dendrocometes paradoxus*, Stein, nebst einigen Bemerkungen über *Spirochona gemmipara* und die contractilen Vacuolen der Vorticellen. Zeitschrift für wissenschaftliche Zoologie, vol. 28, pp. 49-67, plate VI.
1889. Protozoa. Infusoria und System der Radiolaria. Erster Band, III Abtheilung, pp. 1585-2035, plates LVI-LXXIX and 24 woodcuts in text, in Bronn, H. G. Klassen und Ordnungen des Thier-Reichs wissenschaftlich dargestellt in Wort und Bild. Leipzig, C. F. Winter'sche Verlags-handlung.

CANU, EUGÈNE

1886. Note sur le genre *Spirochona* (Stein). Bulletin Scientifique du Département du Nord et des Pays Voisins, vol. 17, pp. 21-31 (no figures); (sometimes listed as Bull. Biol. France Belg., ser. 2, vol. 9).

CLAPARÈDE, ÉDOUARD AND JOHANNES LACHMANN

1858. Études sur les Infusoires et les Rhizopodes. 1^{er}. volume. Institut Genevois, Mémoires, vol. 5, pp. 131-132.

DELAGE, YVES AND EDGAR HÉROUARD

1896. La cellule et les protozoaires. Tome I in Traité de Zoologie Concrète. Paris, Schleicher Freres.

DOFLEIN, FRANZ

1896. Ueber die Kerntheilung bei *Kentrochona Nebaliae*. Zoologischer Anzeiger, vol. 19, pp. 362-366, 5 figs. in text.
- 1897a. Studien zur Naturgeschichte der Protozoen. I. *Kentrochona nebaliae* Rompel. Zoologische Jahrbücher, Abtheilung für Anatomie und Ontogenie der Thiere, vol. 10, pp. 619-641, plates 45-46, text figs. A-L.
- 1897b. *Idem*. II. *Kentrochonopsis multipara* n. g. n. sp., ein Infusor mit multipler Knospung. *Ibid.*, vol. 10, pp. 642-646, plate 47.

EISMOND, JÓZEF

1890. Ueber die Struktur des Peristoms bei Vorticellinen. Biologisches Centralblatt, vol. 10, pp. 255-256.
1895. Studya nad pierwotniakami okolic Warszawy. Pamiętnik Fizyograficzny, vol. 13, pp. 97-227, plates II-V with 44 figs.

ENRIQUES, PAOLO

1908. Di un nuovo Infusorio oligotrico (*Turbilina instabilis* n. gn., n. sp.) e suo significato per la filogenia dei Peritrichi. R. Accademia nazionale dei Lincei, Roma, Classe di scienze fisiche, matematiche e naturali, Rendiconti, ser. 5, vol. 17 (1 semestre), pp. 224-235, 10 figs.

GEEST, B. DE

1933. Protozoaires Rhizopodes-Mycétozoaires-Flagellates-Infusoires. Bruxelles, Les Naturalistes Belges.

GROBBEN, KARL

1905. Lehrbuch der Zoologie. Begründet von C. Claus 7. neubearb. Aufl. Marburg in Hesse, N. G. Elwert.

HAMBURGER, CLARA AND W. VON BUDDENBROCK

1911. Ciliata mit Ausschluss der Tintinnoidea. XIII. in Brandt, Karl and Apstein, C. Nordisches Plankton, Kiel and Leipzig, Lipsius & Tischer.

HENDERSON, W. D.

1905. Notes on the Infusoria of Freiburg in Breisgau. Zoologischer Anzeiger, vol. 29, pp. 1-24, 6 figs.

HERTWIG, RICHARD

1877. Ueber den Bau und die Entwicklung der *Spirochona gemmipara*. Jenaische Zeitschrift für Naturwissenschaft, vol. 11, pp. 149-187, plates X-XII.

HICKSON, SYDNEY JOHN

1903. The Infusoria or Corticata Heterokaryota. [Section L of Part I. Introduction and Protozoa] in Lankester, E. Ray, A Treatise on Zoology. London, Adam and Charles Black.

KAHL, A.

1933. Ciliata libera et ectocommensalia. Lieferung XXIII, Teil II. c. in Grimpe, G. Die Tierwelt der Nord- und Ostsee. Leipzig, Akademische Verlagsgesellschaft.
1935. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) Eine Bearbeitung der freilebenden und ectocommensalen Infusorien der Erde, unter Ausschluss der marinen Tintinnidae. 4. Peritricha und Chonotricha. in Dahl, Friedrich Die Tierwelt Deutschlands und der angrenzenden Meeresteile nach ihren Merkmalen und nach ihrer Lebensweise. Jena, Gustav Fischer.

KENT, WILLIAM SAVILLE

1880. A manual of the Infusoria: including a description of all known flagellate, ciliate, and tentaculiferous Protozoa, British and Foreign, and an account of the organization and affinities of the sponges. [issued in parts, part v, and plate XXXIII, containing the passages on Chonotrichida issued in 1881] London, David Bogue.

KÜHN, ALFRED

1932. Lehrbuch der Zoologie. Begründet von C. Claus 10. neubearb. Aufl. Berlin and Vienna, J. Springer.

[PEARSE, ARTHUR SPERRY, ed.]

1936. Zoological names. A list of phyla, classes and orders, prepared for section F, American association for the advancement of science. Durham, Duke University Press.

PEARSE, ARTHUR SPERRY

1942. Introduction to Parasitology. Springfield, Charles C. Thomas.

PÉNARD, EUGÈNE

1922. Études sur les Infusoires d'eau douce. Genève, Georg & Cié.

PLATE, LUDWIG

1886. Untersuchungen einiger an den Kiemenblättern des *Gammarus pulex* lebenden Ektoparasiten. Zeitschrift für wissenschaftliche Zoologie, vol. 43, pp. 175-241, plates VI-VII.
1888. *Heliochona sessilis* mihi, eine neue Vorticelline. pp. 49-51 in Studien über Protozoen. Zoologische Jahrbücher: Abtheilung für Anatomie und Ontogenie der Thiere, vol. 3, pp. 135-200, plates III-V.

POCHE, FRANZ

1913. Das System der Protozoa. Archiv für Protistenkunde, vol. 30, pp. 125-321, 1 fig. in text.

REICHENOW, EDUARD

1929. Franz Doflein's Lehrbuch der Protozoenkunde usw. II. Teil. Zweite Hälfte: Sporozoa, Ciliata und Suctoria. 5. Aufl. Jena, Gustav Fischer.

RENTSCH, S.

1860. Homoiogenesis. Beiträge zur Natur- und Heilkunde. Erstes Heft. *Gammarus ornatus* und seine Schmarotzer. Wismar, Hinstorff.

ROMPEL, JOS.

1894. *Kentrochona Nebaliae* n.g. n.sp., ein neues Infusor aus der Familie der Spirochoninen, zugleich ein Beitrag zur Lehre von der Kerntheilung und dem Centrosoma. Zeitschrift für wissenschaftliche Zoologie, vol. 58, pp. 618-635, with plate 39.

ROUX, JEAN

1900. Note sur les Infusoires ciliés du lac Léman. Revue Suisse de Zoologie, vol. 8, pp. 459-465.
1901. Faune Infusorienne des eaux stagnantes des environs de Geneve. Mémoires de l'Institut National Genevois, vol. 19, pp. 5-149, 8 colored plates.

SAKOWSKY-CAMPIONI, FRAU —

1907. Verzeichnis der Infusorien der Umgebung von Bern. Mitteilungen der Naturforschenden Gesellschaft in Bern, vol. 1906, pp. 135-137.

STEIN, FRIEDRICH RITTER VON

1852. Neue Beiträge zur Kenntnis der Entwicklungsgeschichte und des feineren Baues der Infusionsthier. Zeitschrift für wissenschaftliche Zoologie, vol. 3, pp. 475-509, plate XVIII.
1854. Die Infusionsthier. auf ihre Entwicklungsgeschichte untersucht. Leipzig, Engelmann.

1859. Der Organismus der Infusionsthierchen, nach eigenen Forschungen in system. Reihenfolge bearbeitet. 1. Abtheil. Allg. Theil und Naturgeschichte der hypotrichen Infusionsthier. Leipzig, Engelmann.

SWARCZEWSKY, B.

- 1928a. Beobachtungen über *Spirochona elegans* n. sp. Archiv für Protistenkunde, vol. 61, pp. 185-222, plates 7 and 8.
1928b. Zur Kenntnis der Baikalprotistenfauna. Die an den Baikalgammariden lebenden Infusorien. V. Spirochonina. *Ibid.*, 64, pp. 44-60, plate 2.

WALLENREN, HANS

1894. Studier öfver Ciliata Infusorier I. Slägtet *Licnophora* Claparède. Lunds Universitets Års-Skrift, vol. 30, no. 6, pp. 1-48, 1 plate and 6 figs. in text.
1895. *Idem.* II. Slägtet *Heliochona* Plate Slägtet *Chilodochona* n.g. Slägtet *Hemispeira* Fabre-Domerque. *Ibid.*, vol. 31, no. 7, pp. 1-72, 4 plates and 10 figs. in text.
1896. Einige neue ciliate Infusorien. Biologisches Centralblatt, vol. 16, pp. 547-556, 8 figs. in text.

PLATE I

Trichochona lecythoides, new genus, new species

- A. Habit sketch of *Trichochona lecythoides* on pleopod of *Amp(h)ithoë* sp., x166.
- B. Mature individual showing marsupium and fully developed funnel, x606.
- C. Juvenile individual, x606.

