SYSTEMATIC DIFFERENTIATION AND EVOLUTION OF FLAGELLATES IN TERMITES

HAROLD KIRBY
University of California, Berkeley.

The population of flagellates in termites is one that interests the physiologist because of the mutualistic relationship essential to nutrition of the host, studied by Cleveland and by Hungate; that interests the student of comparative morphology of protozoa because of the striking characteristics of many of the flagellates; and that interests the student of evolution because circumstances in this association are particularly favorable for considerations of concomitant phylogeny of hosts and symbiotes.

Before giving consideration to some general problems in systematic differentiation and evolution of flagellates in termites. I will discuss some particular groups that are useful as ilustrations. One of those is the genus *Trichonympha,* selected from the hypermastigote flagellates to illustrate species differentiation within the genus. Two large families of trichomonad flagellates in termites, the Devescovinidae and Calonymphidae, will serve as examples of differentiation at that systematic level. The Oxymonadidae, also, show some features pertinent to the discusión. Some general consideration is given to differentiation within the order Hypermastigida, in ilustration of the evolutionary pattern in that characteristic group of termite and roach flagellates.

The genus *Trichonympha* is one of special interest from the standpoint of host distribution and speciation. About twenty-five species are known (Kirby, 1932, 1944; Clevenland *et al.,* 1934). At least five species occur in the roach *Cryptocercus punctulatus,* and the differences between them are as great as between any of the species in termites. The phenotypic features of *Trichonympha* are as complex as in almost any protozoa, so there are many characteristic on which to base systematic treatment. Sexual processes take place in species of *Trichonympha* in *Cryptocercus* (Cleveland, 1947) but not so far as is known in any species in termites.

I have studied *Trichonympha* in fifty-three species of termites, and have myself studied eighteen of the flagellate species. For the most part these species of *Trichonympha* seem to me not to grade into one another in such a way that definition of them is arbitrary or is made consciously or unconsciously under influence of the facts of host distribution. As I have recognized them, the species have well-defilled morphological distinctions.

The parabasal apparatus is a valuable structure for use in systematic study of *Trichonympha.* That apparatus consists of numerous parabasal bodies or cords, generally arranged in a circle around the region of the nucleus and extended by filaments to the region of the base of the rostral tube. Each species has a characteristic and relatively constant size, shape, and arrangement of the parabasal bodies; among species there are notable differences in arrangement. In the most generalized condition, all parabasal bodies are arranged at the same transverse level, and none of them comes into close contact with the nucleus. In many species some or all of the parabasal cords are applied in part to the nuclear membrane, and in some species all of them enter into formation of a basket or pouchlike structure within wich the nucleus lies. There are differences in the individual spiraling of the cords, in their anterior origin where they may all come together in a place near the central longitudinal axis of the flagellate, and in their number and relative length. In two specie s the parabasal cords, instead of originating as is usual all at one transverse level, begin in the peripheral endoplasm at various levels.

Other differences between species of *Trichonympha* may be found in the shape of the rostral tube; the length of the flagellated zone; the length of the flagella; the structure and size of the nucleus; and the presence or abscence of certain endoplasmic inclusions. In regard to the nuclear differencies it is a matter of interest that two species that seem identical with one another in morphology, including body size, differ in that the nucleus of one averages only about half the diameter of that of the other.

In termites, species of *Trichonympha* occur in three of the four flagellate containing families. The family Mastotermitidae consists of only one species of termite, so the absence of *Trichonympha* has no significance. In the Hodotermitidae, *Trichonympha* is widespread, ocurring in *Anacanthotermes*, *Porotermes, Zootermopsis,* and *Hodotermopsis.*

In the Kalotermitidae, *Trichonympha* has been found in almost a quarter of the termites examined, twelve species in about thirty-five hosts. In the Rhinotermitidae, *Trichonymtha* has been found only in members of the genus *Reticulitermes,* in which it is present in every one.

The presence in some instances of the same or closely similar species of *Trichonympha* in all the species of termites of a genus, or in many of them, is a significant fact in distribution. *Trichonympha turkestanica* or flagellates like it have been found in five species of *Anacanthotermes,* all that have been examined (Kirby, 1944). *Trichonympha campanula* occurs in the two or thre species of *Zootermopsis* that are living. *Trichonymtha chattoni* or a similar form has been found in thirteen species of *Glyptotermes,* half those examined. *Trichonympha* of this type, furthermore, is the only sort found in *Glyptotermes;* either it is present or there is no *Trychonympha. Trichonympha agilis* or a similar form occurs in all species of *Reticulitermes.*

Within a genus of termites the species containing *Trichonympha* may be widely separated geographically. Species of *Glyptotermes* with *Trichonympha* of the T. *chattoni* type occur in Australia, Java, Costa Rica, Fiji Islands, Uganda, and the Philippine Islands. T. *chattoni* or similar flagellates occur also in a few other Kalotermitidae in Florida, South Africa and Ceylon. Species of *Reticulitermes with T. agilis* occur in various parts of the United States; in Japan, and in southern Europe. Though only *Trichonympha* is used here as an illustration, other flagellates of the same hosts similarly show a relationship to the systematic affinities of the host rather than to geographical distribution.

It is probable that in this phylogeny those species of *Trichonympha* common to various members of a genus of termites must go back to the origin of that genus or earlier. They have persisted in members of the genus with little or no change as the termite species differentiated. A flagellate of the T. *chattoni* type must have been present in termites before the genus *Glyptotermes* evolved, as it is present also in a few other Kalotermitidae. It has persisted as the only kind of *Trichonympha* in at least most species of *Glyptotermes,* and there has been no secondary acquisition of other species of the flagellates by termites of that group. There has been little or no diferentiation of *Trichonympha* within *Glyptotermes* so far as visible characteristics are concerned. According to Emerson, the present generic diversification of termites probably dates back into the Mesozoic, so that there has been a remarkable stability of these hypermastigote flagellates within the different species of *Glyptotermes.*

Some species of *Trichonympha* are known from only one or a few hosts each, variously placed systematically in the family Kalotermitidae. We do not have enough information to say much about their phylogeny. The general situation in the genus indicates that they probably are also of ancient origin.

Cryptocercus punctulatus is like a termite in its habitat and food. It is closely related to termites genealogically, having been derived from a common ancestor, presumably in the early or middle Mesozoic. There is reason for believing that *Trichonympha* has been passed along directly from host to host in all this line of descent, and is a very ancient genus. It manifests a long-time stability in generic type, as no flagellates are known that are close to *Trichonympha* in generic characteristics.

Trichonympha in *Cryptocercus punctulatus* has sexual processes, with differentiation of separate gametes and fertilization (Cleveland, 1947). These processes occur only in conjuction with the molting periods of the host, and Cleveland contends that the molting hormone is paramount in their causation. It is not known that sexual processes ocur in *Trichonympha* or any other flagellates in termites.

As in all protozoa, and other organisms too, many so-called species of *Trichonympha* and of other flagellates in termites presumably consist of differentiated biotypes, differing from one another in respects that often have not yet teen discovered. Whan a species is distributed through a number of different host species and has for a very long period been isolated in these hosts, it is scarcely believable that differences have not arisen. A long period of isolation must be allowed for even in widely separated colonies of the same host species. As yet no sufficiently refined methods have been used to place consideration of these flagellates on the same basis as that of some other animals and plants in which the nature of species and of infra specific differences has been thoroughly analyzed.

Differences among individuals of a unit population, and between populations of a species in different host species, have of course been recognized. Sometimes there are size differences, sometimes there are differences in degree of development of various organelles. Whan to regard these as non-genetic variants, when to recognize them as genetically different biotypes within a species, and when to consider them sufficient for defining distinct species has been a problem of decision. One must always work with mixed populations of these flagellates. It is not yet possible to use cultured clones. Systematic difficulties are greater in some groups of the flagellates than in others. It has not seemed feasible at present to differentiate named subspecies, varieties, or forms in these

flagellates. When a recognizable and constant structural difference exists, it has been regarded as sufficient for differentiating species. When designated on that basis, most species can he given diagnoses at least as precise as in other protozoa.
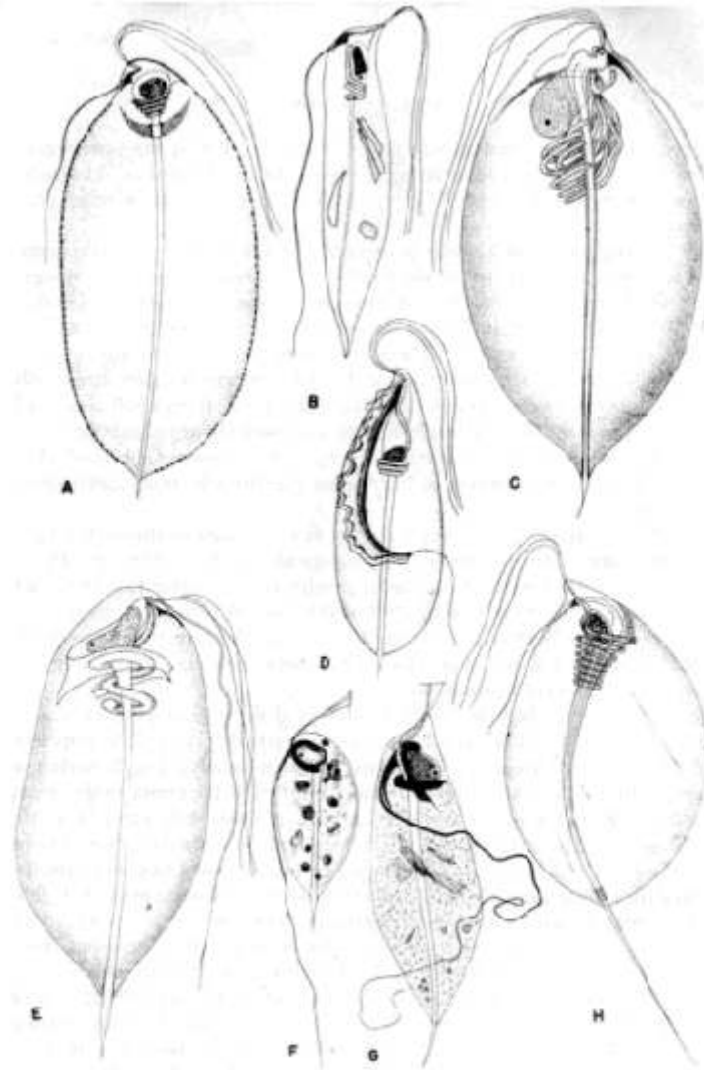

[Plate 1.

Flagellates of the subfamily Devescovininae.
A. *Caduceia kofoidi* Kirby from *Glyptotermes guianensis*, British Guiana. B. *Devescovina camuta* Kirby from *Neotermes* sp. n., El Salvador. C. *Pseudodevescovina ramosa* Kirby from *Neotermes* sp. n., Tanganyika. D. *Macrotrichomonas directa* Kirby from *Kalotermes* sp. n., El Salvador. E. *Metadevescovina magna* Kirby from *Kalotermes marginipennis*, México. F. *Foaina gracilis* Janicki from *Neotermes connexus*, Hawaii. G. *Foaina hilli* (Duboscq & Grassé) from *Glyptotermes iridipennis*, Australia. H. *Hyperdevescovina kalotermitis* (Nurse) from *Proglyptotermes brouni*, New Zealand.
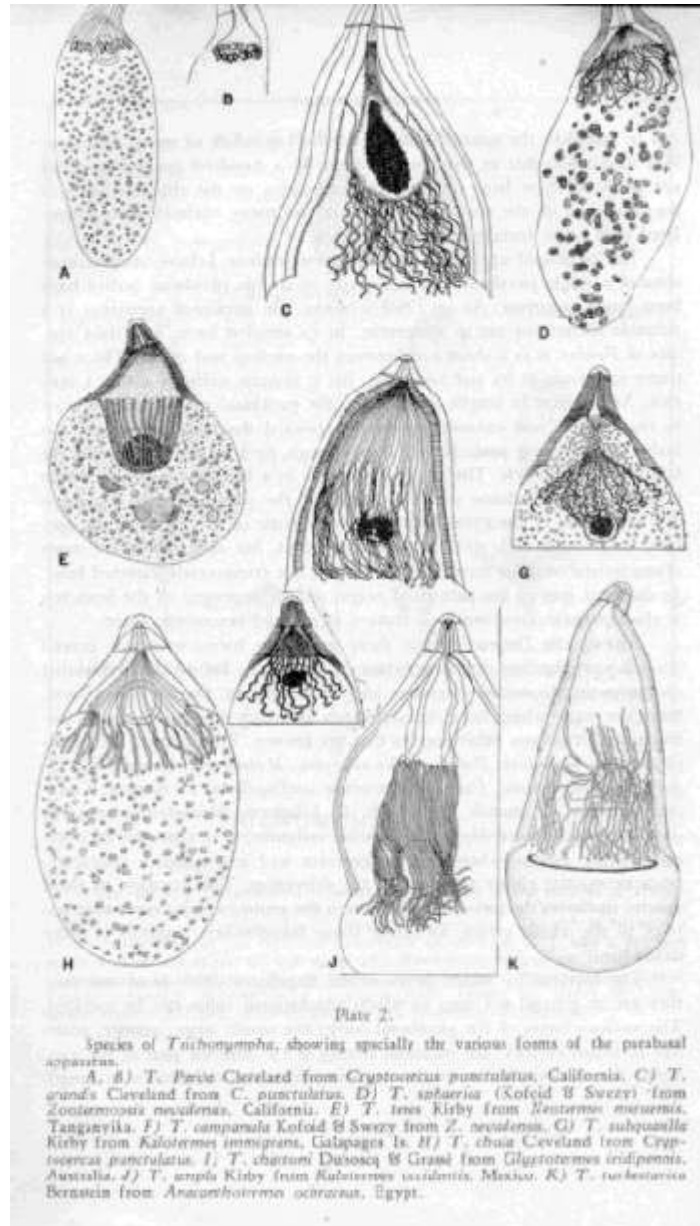
I have given much study to comparative morphology and taxonomy in trichomonad flagellates of the family Devescovinidae, and have described all devescovinid flagellates that occur in about 150 termites (Kirby, 1941-49, 1946). The subfamily Devescovininae is represented in all but eight of 130 kalotermitidae. There are ninety-six species in nine genera. Two of the genera are monotypic, at the other extreme two are large with twenty-six and

twenty species. The flagellates range from small species of *Foaina* of the simplest trichomonad type to complexly organized species of relatively large size. The organelles undergo a development for beyond what has taken place in evolution of trichomona flagellates in any animals other than termites.

The group of three anterior flagella has remained unaltered throughout the family Devescovinidae. The recurrent flagellum varies greatly in size and relative length. It may be as slender as an anterior flagellum, and at the other extreme is a relatively broad ribbon. Within a species its characteristics are relatively constant. In some of the largest devescovinids that are most advanced in development of some organelles the trailing flagellum is relatively small in size.

The cresta which lies in the cytoplasm along at least the firts part of the recurrent flagellum, has undergone an extremely varied development in devescovinid flagellates. It varies much in relative length within a genus. In several genera there are species in which the cresta ranges from small to the full length of the body, and it also shows differences in width. The genus *Macrotrichomonas* is characterized by a marked development of the cresta, wich has become a large, undulatory plate-like structure. In flagellates of the genus *Hyperdevescovina.* which are in some respects highly developed the cresta appears rudimentary. The outer edge of the cresta generally has a spatial relationship to the recurrent flagellum, but there is no correlation in the degree of development of the two structures.

The axostyle in its simplest form is a rod in the longitudinal axis of the body, anteriorly passing along one side of the nucleus. This anterior part may be more or less broadened and flattened as a spoon-shaped structure titted against the nucleus, constituting the capitulum. The capitulum is in some devescovinids remarkably expanded in arms, ridges, or a broad membrane that curves around the anterior part of the body and may bear outgrowths of varied form. Well-defined species characteristics can be found in the manifold shapes of the capitulum of many devescovinid flagellates. But in this group of nearly a hundred species it would not be possible to base supraspecific categories on the characteristics of the capitulum of the axostyle. There are too many variations not correlated with other features of the flagellates.

Plate 2.

Species of *Trichonympha*, showing specially the various forms of the parabasal apparatus.

A, B) *T. parva* Cleveland from *Cryptocercus punctulatus*, California. C) *T. grandis* Cleveland from *C. punctulatus*. D) *T. sphaerica* (Kofoid & Swezy) from *Zootermopsis nevadensis*, California. E) *T. tenes* Kirby from *Neotermes meruensis*, Tanganyika. F) *T. campanula* Kofoid & Swezy from *Z. nevadensis*. G) *T. subquasilla* Kirby from *Kalotermes immigrans*, Galapagos Is. H) *T. chula* Cleveland from *Cryptocercus punctulatus*. I) *T. chattoni* Duboscq & Grassé from *Glyptotermes iridipennis*, Australia. J) *T. acuta* Kirby from *Kalotermes occidentis*, Mexico. K) *T. turkestanica* Bernstein from *Anacanthotermes ochraceus*, Egypt.

The parabasal apparatus in all Devescovininae I have studied consists of a single parabasal body. Reports of double parabasal bodies have been found incorrect. As in *Trichonympha,* the parabasal apparatus is a valuable feature for use in systematic. In its simplest form, in certain species of *Foaina,* it is a short rod between the nucleus and cresta. There are many variations in its size and form, but it is quite uniform within a species. As increase in length takes place, the parabasal may bend posterior to the nucleus and extend transversely toward the opposite side of the body, them turning posteriorly if long enough, or it may spiral around the trunk of the axostyle. The number of gyres in a helix varies some within a species, in accordance with the length of the parabasal, but the shape and separation of the gyres may be characteristic of species. In some species, outgrowths in a spiraled parabasal occur, but outgrowths are more characteristic of those forms in which there is a transversely directed bend. In different species the pattern of origin and arrangement of the branches is characteristic, constituting a feature of marked taxonomic value.

Among the Devescovininae there are many forms in which careful study and evaluation of characteristics is necessary before the systematist can prepare differential diagnoses and define species. On the other hand, there are many which have characteristics by which they can be easily distinguished from any other species that are known. That is true, for example, of *Foaina inflata, Bullanympha silvestrii, Metadevescovina debilis, M. polyspira, M. magna, Pseudodevescovina uniflagellata, P. ramosa, Caduceia bugnioni, C. monile, C. kofoidi, C. kalsoveni, Macrotrichomonas lighti, M. procera,* and *Hyperdevescovina insignita;* it is true also of numerous other species where the smaller size and less complex characteristics necessitate closer attention to the diferences. The situation in these species ilustrates the trend in speciation in the group, which presumably applies to the whole group, including those forms where analysis in more difficult.
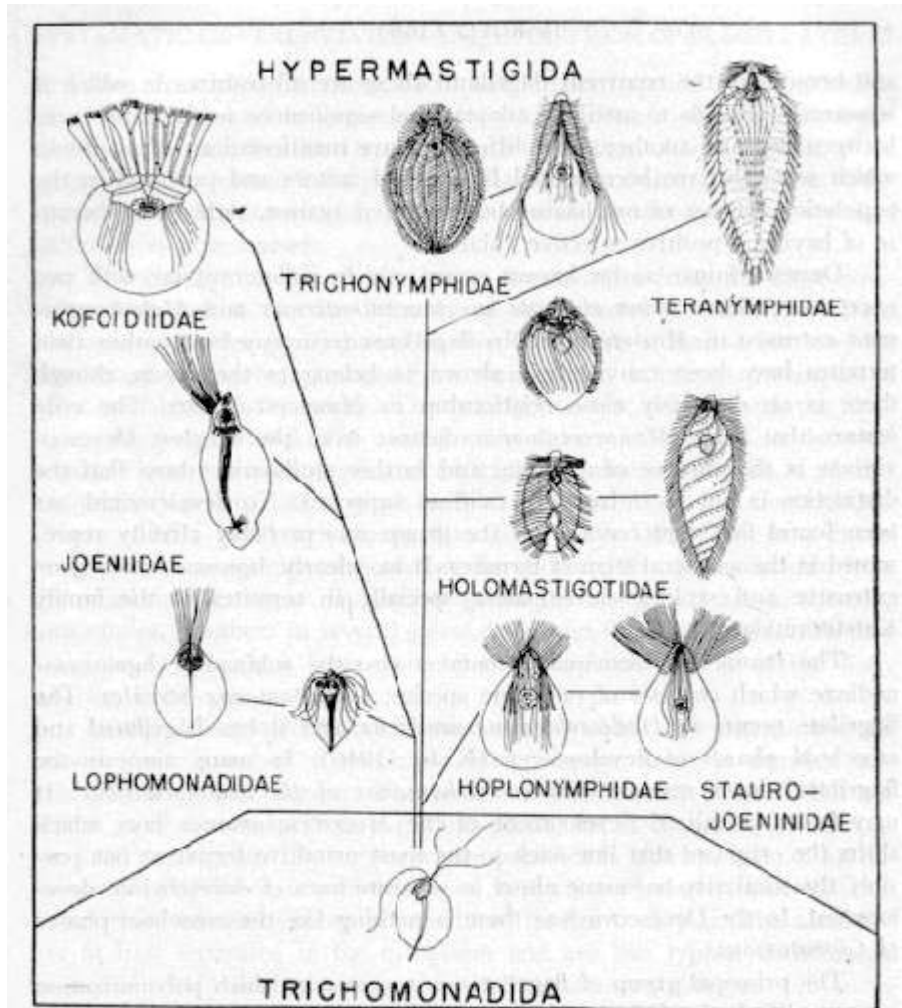

Plate 3.

Chart showing the structural types and relationships in the families of Hypermastigida, with suggested origin from Trichomonadida. On the left is the suborder Lophomonadina, on the right the suborder Trichonymphina. The genera represented (listed in the order of five transverse rows made up of 1, 4, 3, 1, and 4 begining at the bottom and at left of each row) are: *Monocercomonas,* as the basic type of trichomonad; *Lophomonas; Torquenympha, Barbulanympha, Staurojoenina, Joenia, Leptospironympha, Holomastigotes, Euspironympha* (above the figure of *Leptospironympha*), *Kofoidia, Eucomonympha, Trichonympha, Teranympha.*

The features by which devescovinid flagellates differ from one another are in general not ones to which adaptational value can be ascribed. The various forms of the parabasal body; the small, large, slender, scalelike or broad crestas; the manifold shapes of the anterior part of the axostyle; the various forms of the nucleus; even the differences in length and breadth of the recurrent flagellum; these are all features in which it is scarcely possible to attribute adaptational significance to the differences between one and another. The differences are manifestations of evolution which seem to have been caused by internal factors and persisted in the population because of not having been selected against, rather than because of having a positive selective value.

Devescovininae so far known occur only in Kalotermitidae with two exceptions, *Devescovina elongata* in *Anacanthotermes* and *Metadevescovina extranea* in *Mastotermes.* No flagellates from any hosts other than termites have been convincingly shown to belong to the group, though there is an obviously close relationship to *Monocercomonas*. The only feature that keeps *Monocercomonas* distinct from the simplest Desescovininae is the absence of a cresta, and further study may show that the distinction is not so universally valid as supposed. No devescovinid has been found in *Cryptocercus,* but the group was probably already represented in the ancestral stem of termites. It has clearly, however, undergone extensive and striking development specially in termites of the family Kalotermitidae.

The family Devescovinidae contains also the subfamily Gigantomonadinae which consists of only one species, *Gigantomonas herculea.* The flagellate occurs in *Hodotermes mossambicus and* it has flagellated and amoeboid phases of development (Kirby, 1946). In many respects the flagellated form resembles *Macrotrichomonas of the Kalotermitidae.* It may be a specialized development of the *Macrotrichomonas* line, which shifts the origin of that line back to the most primitive termites, but possibly the similarity has come about in separate lines of devescovinid development. In the Devescovininae there is nothing like the amoeboid phases of *Gigantomonas.*

The principal group of flagellates in termites in which polymerization is exemplified, the Calonymphidae, appears clearly to have been evolved from the Devescovinidae. Certain normally monomonad devescovinids are sometimes encountered with two or more mastigont systems, accompanied by nuclei, in a common cystosome. These are only occasional in devescovinid species and are examples of a phanomenon that has been noted in many protozoa. Certain species of *Retortamonas* have been found to occur frequently as double individuals. The tendency toward increase of the number of karyomastigonts without cytoplasmic division has become established as a regular feature in the Calonymphidae. There are eight or nine genera in that group of polymonad flagellates, wich is represented in eighty of 130 kalotermitids of which I have had permanent, stainer preparations. In addition to these, about one hundred species of kalotermitids have been examined in glycerine jelly preparations made from alcoholic specimens of termites in Dr. Emerson´s collection. About half of them were found to contain calonymphids. Flagellates of that family are widespread in the Kalotermitidae, but are restricted to that family of termites so far as is now known.

The Calonymphidae plainly has a direct origin from the Devescovinidae. One species that has been found in a species of *Kalotermes* from Madagascar is like a polymonad devescovinid with a limited but variable number of mastigonts arranged in a manner that is not so precise as in other members of the series. The mastigonts are isolated and the axostyles are not grouped. The mastigont structure is like that in devescovinids. There is a well developed cresta, three anterior flagella, a stouter recurrent flagellum, an axostyle and a parabasal body. In *Coronympha* (Kirby, 1929;1939) the mastigonts are organized precisely in a circle, or eight in one species and sixteen in another, though variations from the usual number occur. In another genus the mastigonts are in three or four circles, in others in several spiral rows, and finally in *Snyderella* they are spread through a large part of the peripheral region of the body (Kirby, 1929).

In the Calonymphidae, as in the Devescovinidae, the anterior flagella remain quite constant throughout, being always three in number and relatively long and slender. The recurrent flagellum is stouter than the others in the more primitive calonymphids, but becomes as slender as the anterior flagella. The cresta, whose presence is characteristic of all devescovinid flagellates, is well developed in the less advanced calonymphids; in the more advanced forms it is reduced in size or is lost. The axostyles are at first separated in the cytoplasm and are like typical trichomonad axostyles with a double-contoured trunk and often an enlarged, projecting posterior end. In the more advanced forms the axostyles are reduced to simple filaments and become grouped in a bundle in the longitudinal axis of the body. This bundle may be organized so compactly as to suggest a single fibrillar axostyle. In the greater number of Calonymphidae, each mastigont has a nucleus associated with it, and may be termed a karyomastigont. In the genera *Calonympha and Snyderella* however, nuclei are fewer than the mastigonts (Janicki,

1915; Kirby, 1929). In the former genus there are both akaryomastigonts and karyomastigonts in the latter the nuclei became spacially dissociated from mastigont stuctures.

A group of polymastigote flagellates which, together with the Devescovininae and Calonymphidae, is a characteristic part of the gut population of termites of the family Kalotermitidae is that of the oxymonad flagellates. They are not closely related to trichomonad flagellates, and have originated separately from the Devescovinidae. The form which is to be found rather widely distributed among animals that most probably is nearly related to the oxymonad flagellates is *Monocercomonoides*. Oxymonadinae are related to the Pyrsonymphinae that occur in *Reticulitermes and* to the genus *Saccinabaculus* in *Cryptocercus punctulatus.* These flagellates constitute a family group, the Pyrsonymphidae, which must have been represented in the ancestral blattoid insects, and has evolved into types now present in *Cyptocercus* and in two groups of termites. Oxymonad flagellates are probably present in almost every species of Kalotermitidae. An account of the subfamily Oxymonadinae was published by Cross (1946), and in her monograph references will be found to earlier work by Janicki, Zeliff and others. Oxymonads occupy a particular habitat in their hosts, adhering to the gut wall, whereas most other flagellates swim freely in the lumen. In most hosts there is a monomonad form of the genus *Oxymonas* with two separated pairs of flagella and a rostellum. Recently de Freitas (1946) described another monomonad form as *Metasaccinobaculus.* Polymerization of mastigont systems has taken place in ways comparable to that in the Calonymphidae, to which the oxymonad flagellates are unrelated. Double individuals of *Oxymonas* are frequently encountered. In *Microrhopolodina* there is a circle of nucleated mastigonts. In *Barroella,* just as in *Snyderella* at the end of the calonymphid series, the nuclei eventually become separated spatially from the mastigont structures. The superficially similar polymonad forms in the oxymonads and calonymphids has sometimes led to an artificial grouping. Actually it is impossible to make a systematic dislocation between monomonad and polymonad Oxymonadinae. Furthermore, the division of polymostigote flagellates into the three categories of monomonad, diplomonad, and polymonad forms is unsound.

Polymerization is exemplified in the hypermastigote flagellates, but in a very different manner from that in Calonymphidae and Oxymonadinae. In those polymastigote groups mastigonts have been duplicated as units, in which the basic pattern has not been altered, and each unit is initially associated with a nucleus. Each unit divides separately; the situation suggests that of a colony of flagellates united by a common cytoplasm. In hypermastigotes there is normally never more than one nucleus and one division figure, but there has been an increase in the number of individual organelles associated with the spindle pole at division.

The flagellate ancestry of hypermastigotes is undoubtedly to be found in trichomonads. There is a parabasal body of the same nature and often an axostyle, and an important indication of affinity exists in the pattern of division. In both groups there is an extranuclear spindle and the cromosomal behavior is similar. But hypermastigotes have diverged greatly from their trichomonad ancestors in estructural organization.

The genera *Prolophomonas* (Cleveland et, al., 1934) and *Torquenympha* (Brown, 1930) consist of hypermastigote flagellates that are relatively simple in organization. *Prolophomonas* has ten to fifteen flagella which originate separately in an anterior circle of basal granules. There is a stout axostylar structure which may consist of agregated filaments. *Torquenympha* is similar, but in it there have been found a number of rounded parabasal bodies suspended separately in the region around the nucleus. Details of the relationship in these flagellates between flagella, blepharoplasts, parabasal bodies, and axostyles are not fully known. They may be thought of as originating by multiplication of the sets of mastigont elements all in relation to a pole of a single spindle. In trichomonads the only comparable increase has been that of anterior flagella, and that has been very limited in scope.

The hypermastigotes may be separated into two divisions which may be designated as suborders, the Lophomonadina and the Trichonymphina; the desirability of that division has been pointed out by Dogiel (1922) and by Grassé et Hollande (1942). In the former suborder there are three families and in the latter five or six. In the *Lophomonadina* the extranuclear organelles are arranged in one group or one system, and in division there is typically renewal of organelles with resorption of the old structures. In the *Trichonymphina* there is basic bilaterality in organization with two or ocassionally four systems of organelles, and in ordinary division there is equal separation of the sets from one another. There is retention, to a greater or lesser extent, of the old structures and a new set forms in each product of division, restoring the paired condition. The poles of the extranuclear spindle may be at some distance away from the actual site of development of the organelles, but there is always a relation to that site by a connecting structure or strand, termed a centriole by Cleveland, which is either a preexisting element in the organization of the flagellate or develops for the purpose of establishing the relationship. Hypermastigotes have generally not taken the further step of complete separation of the organelle systems from relation to the poles of the nuclear division figure, which appears to be a feature in the evolution of ciliates.

The diagram is an attempt to represent the general evolutionary pattern in the Hypermastigida. The series on the left shows the main types of Lophomonadina. Though the group has a unity in certain basic features, there is a great diversity of forms. In the Trichonymphina, on the right, there is also much diversity. In the flagellates of one sort, represented by *Barbulanympha, Hoplonympha, and Staurojoenina,* the flagella are in two or four anterolateral bundles, and within the cytoplasm are two permanent rod-like elements between the posterior ends of which the spindle forms. The rods have been regarded as centrioles (Cleveland); comparable structures exist in all Hoplonymphidae and Staurojoeninidae. The remaining Trichonymphina have flagella in from two to many bands. The bands are more or less steeply spiralled in Holomastigotidae. In *Euspironympha* of *Stolotermes africanus,* which flagellate is related to but apparently separate from the Holomastigotidae, there are two primary spiral bands anteriorly which give rise along their length to numerous flagella-bearing secondary bands. In the Trichonymphidae the bands are numerous and are longitudinal or only slightly spiralled. It is apparent, however, that there is a relationship between the two families Trichonymphidae and Holomastigotidae. *Teranympha* is a form that is in general trichonymphid-like, but body flagella arise from separate circular bands. In it there has been a separation of kinetic elements from direct relation to the center of organization, a unique occurrence in Flagellata.

As a product of evolution in protozoa the flagellates in lower termites and *Cryptocercus* present a notable situation. There are more than fifty genera with over 250 species. Only four genera have species in other hosts. Most of the flagellates are xylophagous, and the environment in which they live is closely comparable from host to host. There seem to be no factors of different nature in the environment that could be supposed to act selectively on the flagellates. Thus some of the most important factors that have been considered to operate in the origin of species seem not to be involved. The organelles in the flagellates are adaptive in their role in vital economy, but the diversity of morphological characteristics which serves for differentiation of genera and species appears to be quite fortuitous.

The characteristics of the different species may be arranged in apparently graded series, but the series in respect to the different characteristics do not correlate with one another. One character remains stationary while another undergoes a degree of elaboration, and there is not infrequently regression. Various organelles in the course of their evolution have displayed fhases of extravagant growth leading to large and overelaborated structures.

So-called determinate series have been noted in various groups of Protozoa in which consideration have been given to evolutionary factors; and in evolution of each of the groups the author has found reason to question the differentiating survival value of the types of differences between species. Metcalf (1928) stated that in evolution of the opalinid ciliates of frogs internal factors are more important than external, and that characters appearing as a result of germinal change seem to go on to greater and greater development, because of conditions in the germ plasm itself. As compared to flagellates in termites, however, the opalinid ciliates show only slight diversity from the simplest to the most advanced form. Trends in development appear in the rich and diversified population of ciliates in the rumen of rumiants. Dogiel (1927) noted in those Ophryoscolecidae a tendency toward progressive increase in size and polymerization of ciliary structures, skeletal plates, and other organelles. In a consideration of the group of lorica-bearing marine ciliates, the Tintinnoinea, which then had 705 species in 51 genera, Kofoid (1930) stated that lines of progressive differentiation are abundantly recognizable. These lines are characterized by size increase, duplication or multiplication of structural features, elongation, and differentiation of surface pattern. The systematic treatment of this group of ciliates is based entirely upon the structure of the lorica. Opalinids, ophryoscolecids, and tintinnids are sexual organisms.

We fail to recognize adaptational significance in many of the differences between characteristics concerned in all those developmental series nor can we find much place for the action of the principle of survival of the fittest. We could range much farther among Protozoa with the same result, though we will not fail to find many instances in which adaptive evolution evidently has played a role. Such are organelles of attachment, to the substratum in fre-living forms, to the wall of the gut in certain endozoic forms. But I hope that in the illustrations made I have not failed to carry the point that it can be only a factor, and furthermore,—at least in certain groups—not the major one. Changes arise in the characteristics of the organisms through internal factor, and the greater part of them are neutral so far as their relationship to the environment goes.

Advantageous variants may undoubtedly occur, particularly in physiological characteristics. Among them would be an enhanced multiplication potencial, an increased capacity for utilizing wood, an ability to withstand unfavorable conditions in transmission, or efficiency in the processes contributing to nutrition of the host. In these physiological characteristics we have at present no means of comparing different species. Evolution of the physiological adaptation between termites and *Cryptocercus* and their xylophagous flagellates is a problem of the greatest interest. But the structural diversification cannot be correlated with that adaptive development.

Flagellates of termites are unlike any flagellates elsewhere, except in certain blattoids, and so are particularly

related to the limited groups of hosts in which they occur. They could have been acquired only from other termites in the lines of descent. That fact makes the group particulary favorable for using the symbiotes as indicators of the relationship of the termites. Concomitant studies of symbiotes and their hosts have sometimes been made a basis for conclusions about centers of origin and geographic distribution, but in this case a more effective opportunity exists for studies of coincidence in phylogeny.

It is only when there are suitable circumstances of restricted transmissibility and host receptivity that concomitant studies in phylogeny of host and symbiote can be dependably undertaken. Symbiotes that have means of dispersal and adaptability which can lead to infection of hosts through ethological rather than phylogenetic relationships do not lend themselves to such studies. Consequently the nature of transmission and host adaptation in termite flagellates is basic to the considerations here adduced.

The flagellates can under experimental conditions be transferred from one termite species to another, provided those host are not too far apart in classification.

Dropkin (1946) found that in mixed colonies of *Kalotermes jouteli* and K. *shwartzi* 44% of the latter and 22% of the former termites acquired some flagellates from the other species, which were than present together with their own faunule. In 11% of *K. shwartzi* only *K. jouteli* flagellates were found. In mixed colonies of *Neotermes castaneus* and *K. jouteli,* 22 of 36 *K. jouteli acquired* at least some *Neotermes* protozoa 5 having only the foreing protozoa. *Neotermes* was less likely to acquire *K. jouteli* protozoa only 5 of 34 having some. Dropkin found that experiments in cross-infection between termites of different families were not successful; protozoa of *Reticulitermes* and *Kalotermes* did not become established in *Zootermopsis (*Dropkin, 1941). Some of the termites in which the faunule consisted partly or entirely of foreing flagellates lived for six months to a year, showing that the foreign protozoa had entered into the mutualistic physiological relationship to the new host.

Cross infection between nearly related termites would not be conspicuously disturbing to the phylogenetic pattern, but it is improbable that it has taken place in nature with possible rare exceptions as will be explained shortly. It is improbable both because of the distribution pattern and of the mechanism of transfer among the termites of a colony and from one colony to another.

Different colonies of a species, though widely separated geographically, do not differ in the qualitative character of the flagellates faunules, except in the occasional absence of one or two species. Only one apparent exception has been found, among many thousands of observations, to the truth of that statement.

A colony of a species collected at Perinet, Madagascar and determined by Emerson as *Neotermes longus* had flagellates different from those of several colonies of *Neotermes longus* collected elsewhere. The flagellates were like those of several colonies of *Neotermes gracilidens* also found at Perinet. Suspecting a confusion in the preparations a careful check was made of determined alcoholic specimens of termites of the colony from which the preparations has been made. The check verified the observations. This instance, alone among a great number of records, seemed to constitute an unexplained exception to the probability of absence of transfaunation in nature. We probably should not deny the possibility, in the many millions of years of termite development, but everything indicates that it has not been an important factor in bringing about the present distribution of flagellates in termites.

The unique phenomena in transfer and maintenace of symbioses in the termite colony is important to the host-specificity of the flagellates, as well as a significant factor in the evolutionary process.

In termites the population of flagellates is lost or reduced at the time of each molt, loss being the usual thing except at last molt. In *Zootermopsis,* as first noted by Andrew and Light (1929), the larval and nymphal molts entail a loss of all the larger xylophagous flagellates; but *Tricercomitus, Hexamastix* and sometimes *Streblomastix* may persist through the molt, often in modified form and numbers. Some of the smaller flagellates may likewise persist through the larval and nymphal molts of various Kalotermitidae but most of the flagellates are lost. In comparable molts of *Reticulitermes lucifugus* all of the flagellates are lost (Grassé et Noirot, 1945).

At the final molt resulting in release of the imago the situation is different. The behavior of protozoa in that molt was studied in several termites by Grassé et Noirot (1945) and earlier by May (1941). May found that in *Zootermopsis* and in *Kalotermes minor* the population of flagellates becomes greatly reduced in number in the final nymphs about to undergo the last molt; but a representative sample of the whole is retained within the saclike shed intima and released on rupture of this into the gut of the imago. Grassé et Noirot showed that there is similar persistance in *Kalotermes flavicollis, Neotermes aburiensis,* and *Reticulitermes lucifugus.* In the Kalotermitids the flagellates are said to pass into cystic or precystic states from which in the winged imago they later transform into active forms (Duboscq et Grassé, 1934; Grassé et Noirot, 1945); but that is not so in *Reticulitermes* according to

Grassé et Noirot.

These observations indicate that only at the last molt are the flagellate in termites continued through the process; after other molts the population must, except for a few small flagellates, be restored by reinfection.

The manner of reinfection has been considered by Imms (1919), Andrew (1930), Goetsch (1936), Grassé et Noirot (1945), and others. All are in agreement that infection is acquired by the ingestion of flagellates passed by the anal opening of another individual. There are no cysts or resistant stages that persist in the outside environment. Infection may also take place by cannibalism. Proctodaeal feeding is a common habit among termites and is the normal method of transfer of the flagellates. It may take place between different species whan these are experimentally mixed (Dropkin, 1946). But owing to the normal antagonism between termites of different species it is so unlikely to happen in nature that cross-infection may probably be ruled out as a factor in explaining the present distribution of the flagellates.

If any cross infection should take place, only infections of foreign flagellates acquired at the last nymphal stage would be significant in the makeup of faunules in a new colony, since the flagellates present in earlier stages are lost at the molts. The likelihood of flagellates from another host infecting that final nymph and persisting among the reduced number carried through to the imago is small, if it exists at all, and the chances would still be overwhelmingly against that imago's being the one that established a new colony, and transmitted the flagellates to the offspring of the pair. The origin and establishment of new species poses a comparable statistical problem. A mutation would be significant only in flagellates present in the last nymphal stage or at the molt, so that it would multiply so as to become an appreciable part of the population from which infection of a new colony might be drawn. Even so, the chances are over whelmingly against the mutant´s ever entering a new colony.

In the present distribution of flagellates in termites, there are numerous instances of limitation of groups of related flagellates to groups of related hosts. It would be possible in many instances, from study of the intestinal protozoa without knowledge of their source, to give the family and sometimes the genus of the termite host. Among known termites of a region, it would often be possible to tell the species. Of 130 kalotermitidae I have examined, I could allocate each one to that family from knowledge of the flagellates. Though the faunule may be quite different in different host, there are certain components that are characteristic. The flagellate faunules of *Reticulitermes* are characteristic of that genus alone among termites. Finding certain flagellates would be a reason for thinking it probable that a termite belongs to the genus *Glyptotermes,* and the flagellates of *Calcaritermes* would definitely place a termite in that genus. Of course, a glance at the gut contents is sufficient to tell whether a termite is one of the Termitidae or not.

There are not a few instances in which flagellates of identical morphology occur in various, and sometimes in all termite species of a genus *Reticulitermes* has similar flagellates in various species, some identical some related; though one species, *R. speratus,* has retained an unusual hypermastigote, *Teranympha mirabilis*, which is absent from the others. Various species of *Anacanthotermes,* in North Africa, Asia Minor, Turkestan, and India contain similar flagellates faunules. *Calcaritermes* of Central and South America has identical flagellates in several species, though there are also specific symbiontes, as *Barroella zeteki in Calcaritermes brevicollis. Barroella* has been retained from older Kalotermitids, as a species occurs also in *Neotermes howa.* Many species of *Glyptotermes,* in various parts of the world, contain *Trichonympha chattoni* and *Macrotrichomonas pulchra.* In certain ones there are forms in which a direct evolution from the more generally distributed type may be traced, as *macrotrichomonas ramosa* in *Glyptotermes brevicaudatus* and *M. unguis* in *G. caudomunitis.*

There are certain species that are widely distributed among hosts of the groups to which they are limited, but occur in some species and not others without reference to the probable lineage. The instances that we have of this are usually in smaller flagellates in which difficulty of analyzing the characteristics is considerable, and in which convergence may not be ruled out as a factor in producing the present species grouping. Such forms are *Devescovina lemniscata* and *D. glabra,* with 19 and 15 geographically scattered kalotermitid host, and three species of *Foaina* with 32, 25, and 21 host in various kalotermitid genera.Unless convergence explains the situation, and it seems unlikely that it can altogether do so, it is necessary to suppose that in various termites these forms have been lost, either by direct elimination or by having given rise to a new form and been replaced by it.

Species that have so wide a host distribution and seemingly are forms of ancient origin and long stability are not in the majority. The greater number of species have been found in a more limited number of hosts, and in various important genera there are many one-host species. Thus, among the Devescovininae the one-host species in *Devescovina* are 9 out of 20, in *Foaina,* 11 of 26, in *Caduceia 4* or 6, in *Macrotrichomonas* 4 of 7, in *Metadevescovina 13* of 15, in *Hyperdevescovina 4* of 6, and the two genera *Parajoenia* and *Bullanympha* are

represented by only one one-host species each. In *Trichonympha* 8 of 18 species in termites are one-host forms, and the five or six species in *Cryptocercus* occur only in that roach.

Sometimes in a termite species, as in *Paraneotermes simplicicormis,* there occur flagellates of unexpected sort, constituting a faunule that in part seems unrelated to that of related termites. Certain flagellates are also present which establish expected relationships, but difficulties arise in explaining the ocurrence of the unusual forms. In *Paraneotermes simplicicornis* there are hypermastigotes that have affinites with flagellates widespread among termites, but usually absent from Kalotermitidae. Certain members of the genera *Neotermes* and *Kalotermes,* which have been placed lower in the kalotermitid scale, have hypermastigotes which are consistently absent from the upper group of genera. Evidently in the origin of that upper group of genera all hypermastigotes except *Trichonympha* early disappeared. Other hypermastigotes, coming through the ancient lineages from beyond the point of origin of *Cryptocercus,* persisted in the ancestors of the lower three genera, but were deleted from the faunules of various hosts.

There is reason for believing that the major part of the generic differentiation of hypermastigotes was completed in the early Mesozoic, or even earlier. These are the most complex of the flagellates; certain groups of the polymastigotes are phylogenetically younger. Hypermastigotes are an ancient group, which in recent times has undergone relatively little evolutionary development; and it is noteworthy that in what appears to be the most actively evolving group of the flagellate-containing termites, the family Kalotermitidae, polymastigotes predominate and not infrequently constitute the whole of the faunule. Hypermastigotes have a lower viability than polymastigotes, and are the first to be lost under unfavorable conditions. Differences in the faunules of individual termites or of different colonies of a species, are found mainly in the presence or absence of certain hypermastigotes; clearly a new species developed from a group in which the form was absent would have lost that flagellate as a component of its faunule.

The taxonomic characteristics of the flagellate population in termites are closely correlated with phylogeny of the insects, and not with geographical distribution. But in these hosts and symbioses there is no correlation of more advanced forms of symbioses with more advanced forms of hosts. Hypermastigotes that are at least as highly evolved as any occur in the hosts that must be regarded as the most primitive of all, *Cryptocercus punctulatus* and *Mastotermes darwiniensis.* Environmental conditions for the flagellates have remained essentially constant in all the members of this group of wood-eating insects, and the time and circumstances for evolutionary change have been equal whether or not the host has retained more primitive characteristics.

Since Lespes in 1856 discovered the protozoa of termites, a large number of investigators in many countries have published descriptions and cytological studies, and some have done important experimental work. Out of the data, it is becoming possible to construct a framework for understanding of the flagellates and their host relationships. Much more accurate study is needed to fill in this framework before it will be possible to make a consistent and convincing analysis of the situation. Most conclusions about the probabilities are to a large exent tentative at this time, and revision will undoubtedly be necessary as more work is done. Studies of the faunules of flagellates should always be related to determinable specimens from the colonies of the hosts (soldiers or winged forms) so that correlation will be possible with termite phylogeny now being explored by Professor A. E. Emerson of the University of Chicago, whatever changes ensue in the taxonomy of the termites. Careful descriptive and experimental work in this field will contribute materially to solution on of a significant and interesting problem in biology.

LITERATURE CITED

ANDREW, B. J. 1930. Method and rate of protozoan refaunation in the termite *Termopsis angusticollis* Hagen. Univ. Calif. Publ. Zool., 33: 449-470.

ANDREW, B. J., and S. F. LIGHT. 1929. Natural and artificial production of so-called "mitotic flares" in the intestinal flagellates of *Termopsis angusticollis.* Univ. Calif. Publ. Zool., 31: 433-440.

BROWN. V. E. 1930. Hypermastigote flagellates from the termite *Reticulitermes: Torquenympha octoplus* gen. nov., sp. nov., and two new species of *Microjoenia.* Univ. Calif. Publ. Zool., 36: 67-80.

CLEVELAND, L. R. 1923. Symbiosis between termites and their intestinal protozoa. Proc. Nat. Acad. Sci., Wash., 9: 424-428.

— 1926. Symbiosis among animals with special reference to termites and their intestinal flagellates. Quart. Rev. Biol., 1: 51-60.

— 1947. Sex produced in the Protozoa of *Cryptocercus* by molting. Science, 105: 16-18.

CLEVELAND, L. R., HALL, S. R., SANDERS, E. P., and COLLIER, J. 1934. The wood-feeding roach *Cryptocercus,* its protozoa, and the symbiosis between protozoa and roach. Mem. Acad. Arts Sci., 17:i-x, 185-342.

CROSS, J. B. 1946. The flagellate subfamily Oxymonadinae. Univ. Calif. Publ. Zool., 53: 67-162.

DOGIEL, V. A. 1922. Untersuchungen an parasitischan Protozoen aus dem Darmkanal der termites. III. Trichonymphidae. Arkh. russk. Protist. Obshch., 1: 172-234.

— 1927. Monographie der Familie Ophryoscolecidae. Teil I. Arch. Protistenk., 59:1-288.

— 1929. Polymerization als ein Prinzip der progressiven Entwicklung bei Protozoen. Biol. Zbl., 49: 451-469.

DROPKIN, V. H. 1941. Host specificity relations of termit eprotozoa. Ecology, 22: 200-202.

— 1946. The use of mixed colonies of termites in the study of host-symbiont relations. J. Parasit., 32: 247-251.

FREITAS, GILBERTO DE. 1946. Sobre a morfologia e ciclo evolutivo dos flagelados do genero *Metasaccinobaculus* n. gen. (polymastigina, Oxymonadidae) do termita *Kalotermes (Neotermes) Wagneri,* Desneux, 1904, com a descricao de duas espécies novas. Mem. Inst. Osw. Cruz, 43 : 349-378.

GOETSCH, W. 1936. Beiträge zur Biologie des Termitenstaates. Z. Morph. Okol. Tiere, 31: 490-560.

GRASSÉ, P. P. et ANDRÉ HOLLANDE. 1934. Les affinités et l'évolution des Trichonymphines. C. R. Acad. Sci., Paris, 215: 35-37.

GRASSÉ, P. P. et CHARLES NOIROT. 1945. La transmission des Flagellés symbiotiques et les aliments des termites. Bull. Biol., 79: 273-292.

HUNGATE, R. E. 1938. Studies on the nutrition of *Zootermopsis.* II. The relative importance of the termite and the Protozoa in wood digestion. Ecology, 19: 1-25.

— 1939. Experiments on the nutrition of *Zootermopsis.* III. The anaerobic carbohidrate dissimilation by the intestinal protozoa. Ecology, 20: 230-245.

IMMS, A. D. 1919. On the structure and biology of *Archotermopsis,* together with descriptions of new species of intestinal protozoa, and general observations on the Isoptera. Phil. Trans. Roy. Soc. (B), 209: 75-180.

KIRBY, HAROLD, 1929. *Snyderella* and *Coronympha.* two new genera of multinucleate flagellates from termites. Univ. Calif. Publ. Zool., 31: 417-432.

— 1932. Flagellates of the genus *Trichonympha* in termites. Univ. Calif. Publ. Zool., 37: 349-476.

— 1939. Two new flagellates from termites in the genera *Coronympha* Kirby and *Metacoronympha* Kirby, new genus. Proc. Calif. Acad. Sci., 22: 207-220.

— 1941-1949. Devescovinid flagellates of termites. Univ. Calif. Publ. Zool., 45: 1-422.

— 1944. The structural characteritics and nuclear parasites of some species of *Trichonympha* in termites. Univ. Calif. Publ. Zool., 49: 185-282.

— 1946. *Gigantomonas herculea* Dogiel, a polymastigote flagellate with flagellated and amoeboid phases of development. Univ. Calif. Publ. Zool., 53:163-226.

— 1947. Flagellate and host relationships of trichomonad flagellates J. Parasit., 33: 214-228.

KOFOID, C. A. 1930. Factors in the evolution of the pelagic Ciliata, the Tintinnoinea. Contributions to Marine Biology, 1-39. (Stanford University Press).

METCALF, M. M. 1928. Trends in evolutions: a discussion of data bearing upon "orthogenesis". J. Morph., 45: 1-45.