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Harold Kirby's Symbionts of Termites: Karyomastigont Reproduction and Calonymphid Taxonomy

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Abstract

Harold Kirby's brilliant principle of mastigont multiplicity is published here posthumously more than 40 years after it was written. He applies this principle to large multinucleate protist symbionts of termites in establishing the taxonomy of Calonymphids (Family Calonymphidae in Phylum Zoomastigina, Kingdom Protoctista). The nuclei and kinetosomes in these heterotrophic cells are organized into trichomonad-style mastigont units which reproduce independently of cytokinesis to generate nine new *Calonympha* and nineteen new *Stephanonympha* species. The total of six genera (*Calonympha, Coronympha, Diplonympha, Metacoronympha, Snyderella* and *Stephanonympha*, all symbionts of dry-wood-eating termites, Kalotermitidae) are recognized. With the aid of Michael Yamin, the distribution of all twenty-eight of Kirby's *Calonympha* and *Stephanonympha* species are tabulated. In italic type I have annotated this paper to be comprehensible to a wide readership of cell biologists, protistologists and those interested in insect symbionts. Although this extremely original and careful work was not finished when Kirby died suddenly in 1952, I deemed it important and complete enough to finally publish it so that it would not be lost to scientific posterity.

Keywords: centriole-kinetosome DNA, karyomastigonts, undulipodia, *Calonympha, Stephanonympha*, termite protists, Kalotermitidae

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* 1900–1952. We dedicate this paper to the memory of Dr. Lewis Thomas (1913–1993) who wrote the foreword to the *Handbook of the Protoctista* (see p. 62.)

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1. Introduction

This manuscript was nearly finished by Harold Kirby, Professor of Zoology at the University of California, when he died in November 1952. A heart attack while leading a Boy Scout expedition in the Sierra Nevada Mountains cut short a life time of work of one of the last protozoologists. Now more than forty years after his death, the uniqueness and quality of his observations inspired me to complete the manuscript for publication. A biography of Professor Kirby excerpted from the Journal of Parasitology, *as well as commentary on this posthumous publication and its terminology begin on page 58. Even though Kirby aided in the establishment of the Society of Protozoologists, no obituary was published by its* Journal of Protozoology, *since that journal was not founded until 1954.*

Kirby's prose, except for my alteration of the terms noted, is in roman type. My figures–illustrations by Kathryn Delisle based on electron micrographs and drawings–are referred to by Arabic numerals, and Kirby's, as in his original plates, are by Roman numerals. The first four pages of his manuscript were taken from an oral presentation; neither discussion nor abstract were even drafted. What you are reading, therefore, is necessarily somewhat disjointed and incomplete; however, I deem it far better for the scientific community to have Kirby's research, representing a culmination of years of previous work, than for this profound, competent study of calonymphids to be lost to science.

When Professor Bronislaw Honigberg of the University of Massachusetts Zoology Department died on March 16, 1992, Kirby's unpublished manuscript and the negatives of the illustrations were found among his scientific materials by his widow, Rhoda Honigberg. The original manuscript, figure plates, and fixed and stained specimens on slides were deposited in 1992 by Dr. Michael Yamin [Vice President and Scientific Director, Alteon, Inc., 165 Ludlow Avenue, Northvale, NJ 07647,] in the collections of the American Museum of Natural History. Details such as numbers of type-specimen slides have been removed from this text; they are available on request. Dr. Kumar Krishna of the Department of Entomology of the American Museum of Natural History, 79th Street and Central Park West, New York City, has the termites, but the calonymphid type specimens and many others of Kirby's slide collection are housed in the Department of Invertebrates, Dr. R.T. Schuh, curator. The calonymphids and other Kirby materials were brought to the museum by Dr. Gregory Hinkle and Professor John Lee after Honigberg's death.

My aim here is simply to share Kirby's remarkable insights with the modern readership. I have conformed the terminology to that of the Handbook of Protoctista *(Margulis et al., 1990) and the* Illustrated Glossary of Protoctista

(Margulis et al., 1993) for reasons of logic. Definitions of specialized terms used in this work begin on page 58. References cited in my italicized portions of this text begin on page 61, whereas Kirby's bibliography begins on page 57. The explanations or alternative terms between square brackets are mine. The question marks are in Kirby's original manuscript.

Mastigonts are organellar systems found primarily in termite symbionts, amitochondrial mastigotes in anoxic conditions. Examples of the mastigont systems of trichomonads are depicted in Fig. 1. They are composed of nuclei (karyomastigonts) to which are often attached parabasal bodies (Golgi apparatus), kinetosomes and their undulipodia, crestae or costae and sometimes other features. These organelle systems (karyomastigonts) increase in number by reproduction and subsequent failure of cytokinesis. The protists bearing the differing numbers of karyomastigonts are related by descent (Honigberg and Brugerolle, 1990). Karyomastigont organellar systems that lack the nucleus are called akaryomastigonts. Both karyomastigonts and akaryomastigonts occur in the same protist. The increase in number of both karyomastigonts and akaryomastigonts, Kirby calls "mastigont multiplicity." The calonymphid series is the most prominent; devescovinids are the basal members of the calonymphids. Devescovinids have a single mastigont system–i.e., nuclei and associated organelles. Giant calonymphids, the largest members of the series, as in Stephanonympha spinosa, *display polysemes: They may have up to 300 mastigonts–i.e., kinetid units–with (karyo-) and without (akaryo-) nuclei in common cytoplasm. In the calonymphid series the number of akaryomastigonts per cell tends to be larger than the number of karyomastigonts. All calonymphids have more than two karyomastigonts. So far, apparently all calonymphids are symbiotic in dry-wood-eating termites, Isopterans of the family Kalotermitidae. Although of large size, calonymphids are an obscure group of protists. Large calonymphids having both akaryomastigonts (without nuclei) and karyomastigonts (with nuclei) in the same cell are ideally suited to the search for kinetosome-centriole DNA (Hall et al., 1989) unfettered by confusion with nuclear DNA. With the exception of Brugerolle's thesis (1976), Lavette (1971), work summarized in the first volume of the* Treatise of Zoology *(Grassé, 1952), and in Lee et al. (1985), Kirby's paper seems to be the only new publication on the family Calonymphidae (Grassi and Foà, 1911) since just prior to his death (Grassé and Hollande, 1951).*

2. Multiple Organization in Mastigotes

Kirby's introductory statement on multiple organization, his observation of many karyomastigonts per protist cell, follows. Marked in handwriting, we

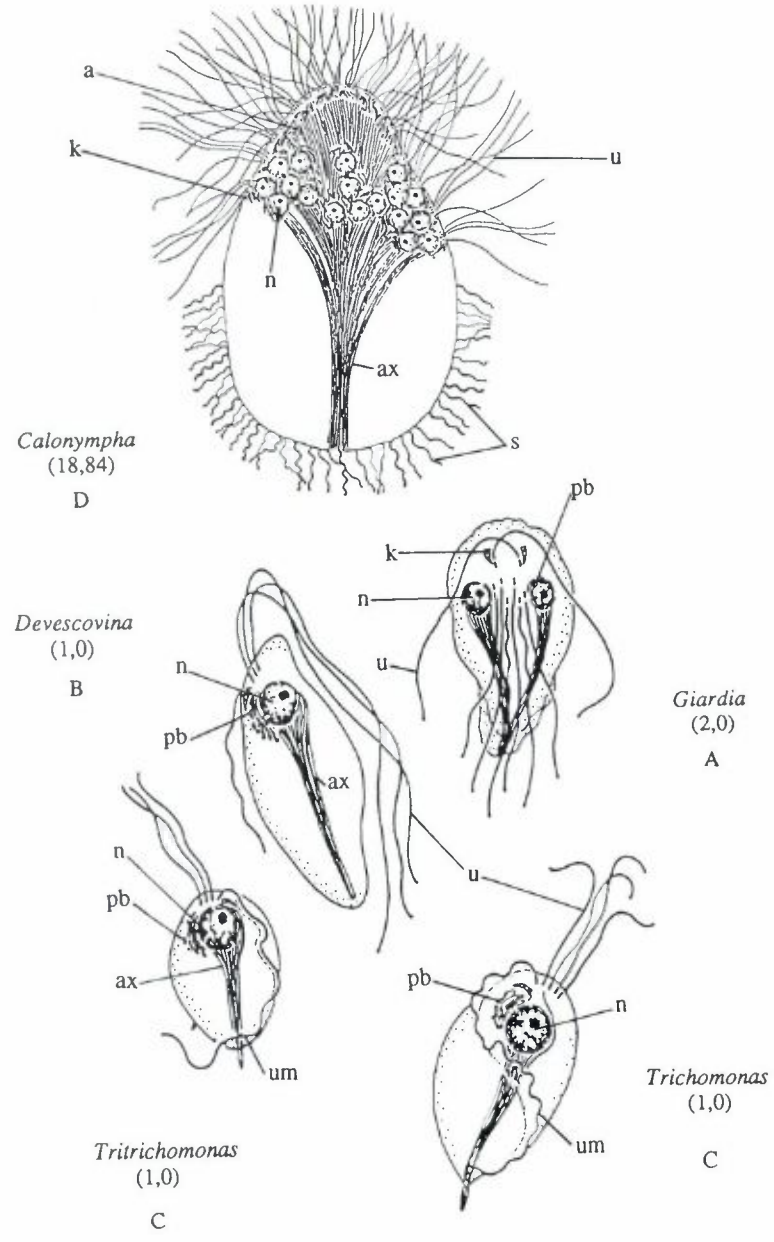
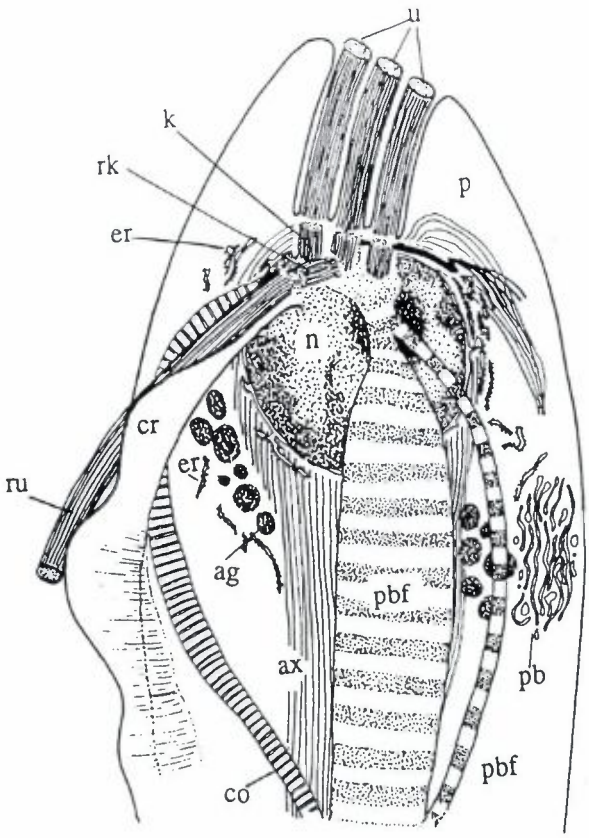


Figure 1. a. Karyomastigonts of zoomastiginids ordered by number of karyomastigonts, relative to number of akaryomastigonts from most to fewest: *Calonympha angusta* (3–75, 10–200); *Giardia* (diplomonad, 2,0); *Devescovina* (1,0); *Tritrichomonas* (1,0).
See page 11 for classification A-D and key (Drawings by Kathryn Delisle).



KEY:
PHYLUM ZOOMASTIGINA

A Class Diplomonadida

Family Hexamitidae

Subfamily Giardiinae

Class Parabasalia

Order Trichomonads

B Family Devescoviidae

C Family Trichomonadidae

D Family Calonymphidae

Figure 1.b.

ABBREVIATIONS: Reconstruction of a generalized trichomonad karyomastigont, based on electron micrographs. (Figures 1–3): ax = axostyle; ag = axostyle granules (probably hydrogenosomes); co = costa; cr = cresta; er = endoplasmic reticulum; k = kinetosome; n = nucleus; p = pelta; pb = parabasal body; pbf = parabasal filaments; rk = kinetosome of recurrent, or trailing, undulipodium; ru = recurrent undulipodium; s = adhering spirochetes; u = undulipodium; um = undulating membrane.

believe it was given as a talk to the American Society of Protozoologists on December 20, 1951 and that he planned to use it as his introduction to this entire manuscript.

In most mastigote protists there is a single nucleus and a single mastigont system. The mastigont system may be simple, consisting of one or two undulipodia and kinetosome or kinetosomes, as in chrysomonads, oikomonads, and monads; or it may be made up of various organelles, as in trichomonads [Fig. 1b]. One type of multiple organization in mastigotes is increase in the number of certain parts of the mastigont system, primarily of undulipodia and kinetosomes, while the nucleus remains single. Multiplicity of that type exists in the chrysomonad *Didymochrysis*, which has two sets of two undulipodia each, and it occurs in various forms in polymastigote[s] and hypermastigote[s]. It is a feature also of zoospores of certain algae, notably *Derbesia* and *Oedogonium*. Organelle multiplicity is of basic importance in evolution of mastigotes and ciliates. It is, however, another type, mastigont multiplicity, that I will discuss in this paper.

Mastigont multiplicity is multiple organization that amounts to the existence of two or more complete mastigote units in a common cytoplasmic body. It gives the types known as diplomonad and polymonad mastigotes. Each unit consists originally of a nucleus and a mastigont system, though in more advanced development the mastigont systems outnumber the nuclei [increasing the ratio of akaryomastigonts to karyomastigonts (Fig. 1a)]. Such multiple organization is seldom found in the pigmented groups of mastigotes, where it does exist in the dinomastigote *Polykrikos*. It exists also in the zoospore of *Vaucheria*. In the nonpigmented groups it occurs in the hexamitids, oxymonads, and calonymphids. Mastigont multiplicity has often been regarded as a reason for dividing the group of polymastigote mastigotes into three major taxonomic categories: the Monozoa, Diplozoa, and Polyzoa; or monomonads, diplomonads, and polymonads. That, however, is an artificial taxonomic procedure that fails to recognize natural affinities.

In the hexamitids the double organization is somewhat specialized, and direct evidence of the monomonad relatives is lacking. In the oxymonads and calonymphids, however, the immediate origin of the multiple forms is clearly apparent, and their relationship to monomonad forms is obvious. The polymonad oxymonads and the calonymphids have no close affinity to one another, as is true of the monomonads of these two types.

Calonymphids are trichomonad mastigotes, in the wide sense [compare Fig. 2, lower right, with Fig. 1b]. That is shown by comparison of their

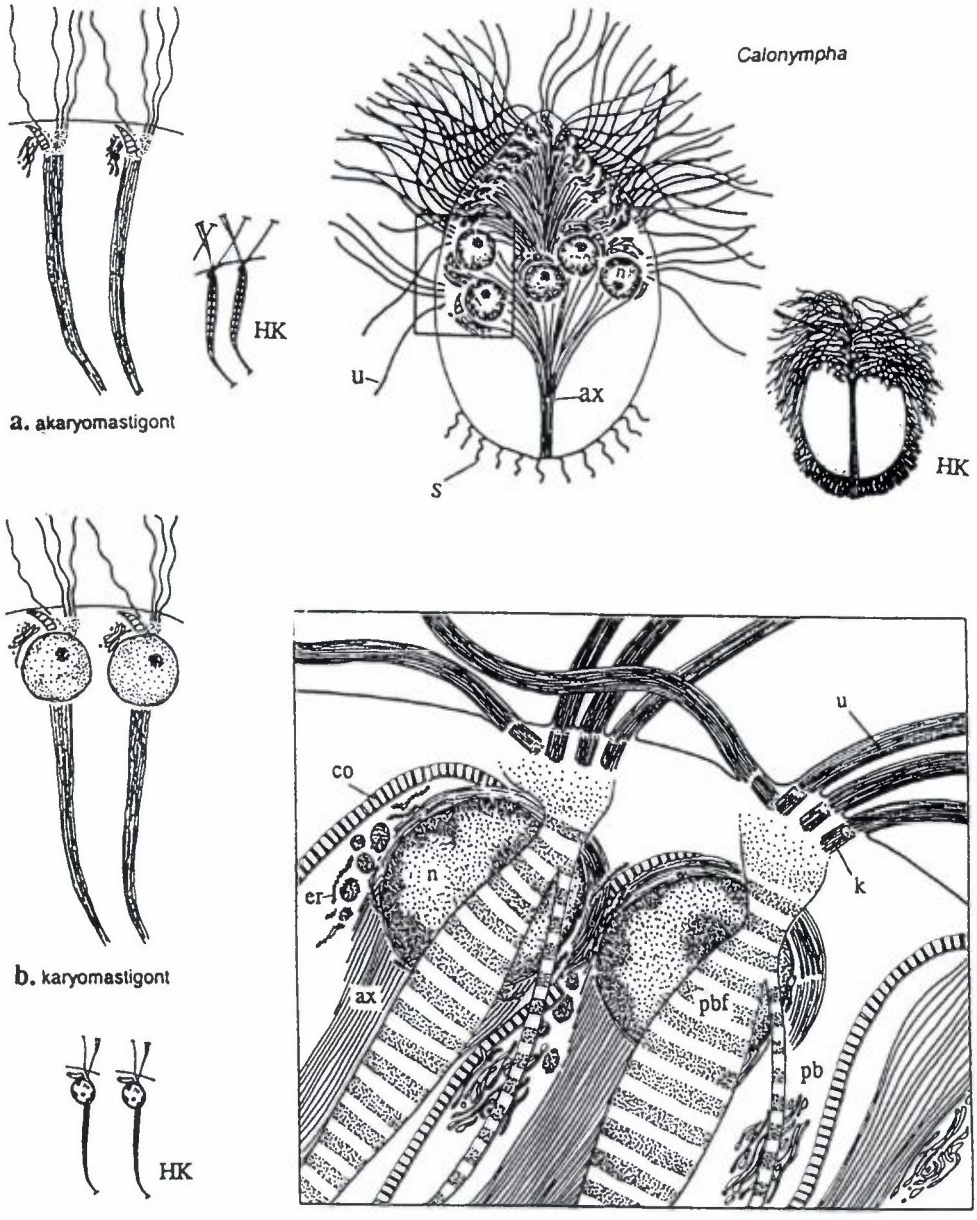


Figure 2. Karyomastigonts compared with akaryomastigonts. Generalized structures at left, which interpret Kirby's reduced drawings (HK) of akaryomastigonts (a) and karyomastigonts (b). *Calonympha* sp. at top right was interpreted from Kirby's reduced drawings and electron micrographs. The two karyomastigonts in the box are shown enlarged below as reconstructed from electron micrographs. The related akaryomastigonts are depicted in Fig. 3.

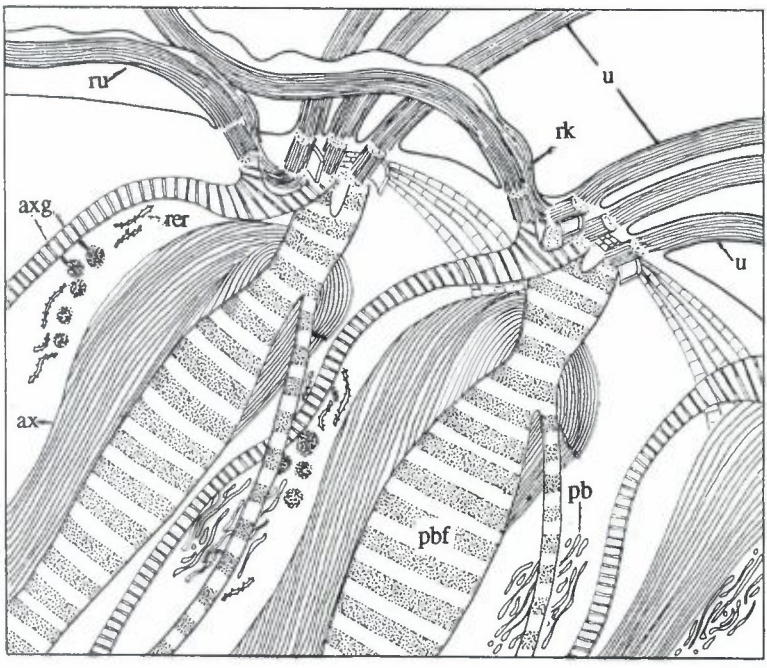


Figure 3. Two calonymphid akaryomastigonts as reconstructed from Brugerolle's electron micrographs, Mignot's drawing (Brugerolle, 1976), and Kirby's text. (Drawings by Kathryn Delisle.)

mastigont system with that of *Monocercomonas, Devescovina*, and *Trichomonas* and by comparison of the type of mastigont division in the polymonad and monomonad forms. The particular combination of anterior undulipodia, recurrent undulipodium, costa or cresta, axostyle, and parabasal body exists in no other mastigotes [Fig. 1b; 3]. Any supposed departure from this combination of structures should be investigated carefully. Published statements of the absence of the axostyle in certain forms have been shown fallacious. The parabasal body has been supposed to be absent in some, but that has generally been only a failure in the technique of demonstration. It has been found in every trichomonad I have examined, with a single exception, *Gigantomonas herculea*. The costa or cresta is in most groups of trichomonads so characteristic that its supposed absence in members of those groups may be doubted. The recurrent undulipodium is always present, either free or adherent in an undulating membrane. This constancy of type includes also the anterior undulipodia. A species of trichomonad has a characteristic number [of parabasals], and departure from that number is in the category of an abnormality rather than a normal variation.

Figure 4. Harold Kirby (1900–1952); c. 1950, in his University of California, Berkeley, office. (Courtesy of Michael A. Yamin.)

The mastigont in calonymphid mastigotes is like that in devescovinid mastigotes, and in some the degree of development of all the organelles is comparable to that of some devescovinids. In others certain organelles are reduced, but they do not disappear. The parabasal body in the apical akaryomastigonts of some species of *Calonympha* is a minute granule less than 0.5 μm in diameter. The cresta in certain species of *Stephanonympha* and *Calonympha* is reduced to a minute structure no more than 1 μm in length. The arrangement of undulipodia in calonymphids is the same as in monomonad trichomonads. Three anterior undulipodia extend free, and the fourth runs separately along the cresta, just as in devescovinids.

The pattern of division is identical in all trichomonad mastigotes. As in all of them, in the calonymphids there is an extranuclear spindle, the axostyles

are discarded and new ones grow out, and the old parabasal at least in part is retained, with a new one developed at the other pole of the spindle.

Devescovinids are monomonads, but one finds at times specimens in which delayed cytoplasmic division results in the presence of a number of mastigonts in a common cytoplasmic body. That abnormality, which happens in various trichomonads, shows directly how the calonymphids originated. Division of the cytosome [cytokinesis], which in most trichomonads follows or accompanies mastigont division, has become separated from it in the calonymphids. In fact, it never occurs in the same form in those polymonads; in them, all the mastigont units become dispersed and divide simultaneously. Then they organize into two groups, and cytoplasmic division separates the groups. No monomonad mastigotes exist in the calonymphid group.

Comparative studies of multiple organization in nonpigmented mastigotes have brought out the following points:

1. Mastigont multiplicity occurs in several independent groups of mastigotes.
2. Calonymphids are the result of mastigont multiplicity developed in the trichomonad series. The organelles, even though vestigial in some instances, correspond to those of devescovinids and other trichomonads.
3. Trichomonad mastigonts, in both monomonad and polymonad forms, show a high degree of constancy in makeup and in pattern of division.
4. Oxymonad mastigotes differ markedly from trichomonads in mastigont makeup and pattern of division. There is a polymonad series of oxymonads, which is not directly related to that of trichomonads.
5. In each group, the multiple forms should be related to the forms from which they originated, and not included in a common category, separated from their monomonad relatives, by reason of the multiplicity.

3. Results: Descriptions and Drawings

The rest of the manuscript found in draft form follows. The original fixed and stained microscopic preparations were given to Michael A. Yamin by Kirby's artistic assistant in Berkeley, California, the talented illustrator Lois Stone, in the late 1970s. The original slides and plates have been forwarded to the American Museum. The entire Calonymphidae collection eventually should be merged and properly archived as is intended by the Dept. of Invertebrates of the museum.

Plates

All drawings were made with the aid of the camera lucida. Relative sizes of objects are shown by a scale bar on each plate. We [Lois Stone and Harold Kirby] used the same optical equipment in making these illustrations so that size variations between species might be more evident.

Plate I

(a) *Stephanonympha silvestrii* from *Neotermes connexus*; (b) mastigont detail of *S. silvestrii*; (c) detail of parabasal bodies and nuclei showing variation in size from anterior to posterior parabasals; (d, e, f) various mastigont patterns of *S. silvestrii*; (g) *Stephanonympha grassii* from *Cryptotermes havilandi*; (h) detail of mastigont in *S. grassii*; (i) nucleus with parabasal body of [*S. grassii*].

Plate II

(a) *Stephanonympha nelumbium* from *Cryptotermes dudleyi*; (b) mastigont detail of *S. nelumbium*; (c) parabasal bodies with nuclei of *S. nelumbium*; (d) posterior portion of *S. nelumbium* showing bundle of axostyles and spherical body; (e) spherical body containing dark areas; (f) spherical body lacking dark areas; (g, h, i) various types of terminal axostyle portions of bundles.

Plate III

(a) Mastigont detail of *Stephanonympha expleta* from *Neotermes erythraeus*; (b) *Stephanonympha diversa* from *Cryptotermes* sp.; (c) mastigont detail of *S. diversa* showing varying shape of parabasal body; (d) detail of *S. diversa* mastigont showing cresta; (e) anterior portion of *Stephanonympha spinosa* from *Cryptotermes queenslandi* showing cresta; (f) mastigont detail of *S. spinosa*; (g) detail of nuclei and parabasal bodies in *S. spinosa*; (h) *Stephanonympha rotunda* from *Cryptotermes cavifrons*; (i) detail of parabasal body of *S. rotunda*; (j) mastigont detail of *S. rotunda*.

Plate IV

(a) *Stephanonympha rarita* from *Neotermes greeni*; (b) mastigont detail from *S. rarita*; (c) detail of parabasal body in *S. rarita*; (d) nuclei and parabasal bodies of *Stephanonympha assimilis* from *Cryptotermes kirbyi*; (e) *Calonympha grassii* from *Cryptotermes brevis*; (f) mastigont detail from *Calonympha grassii*; (g) detail to show granules in nucleus of *Calonympha grassii*.

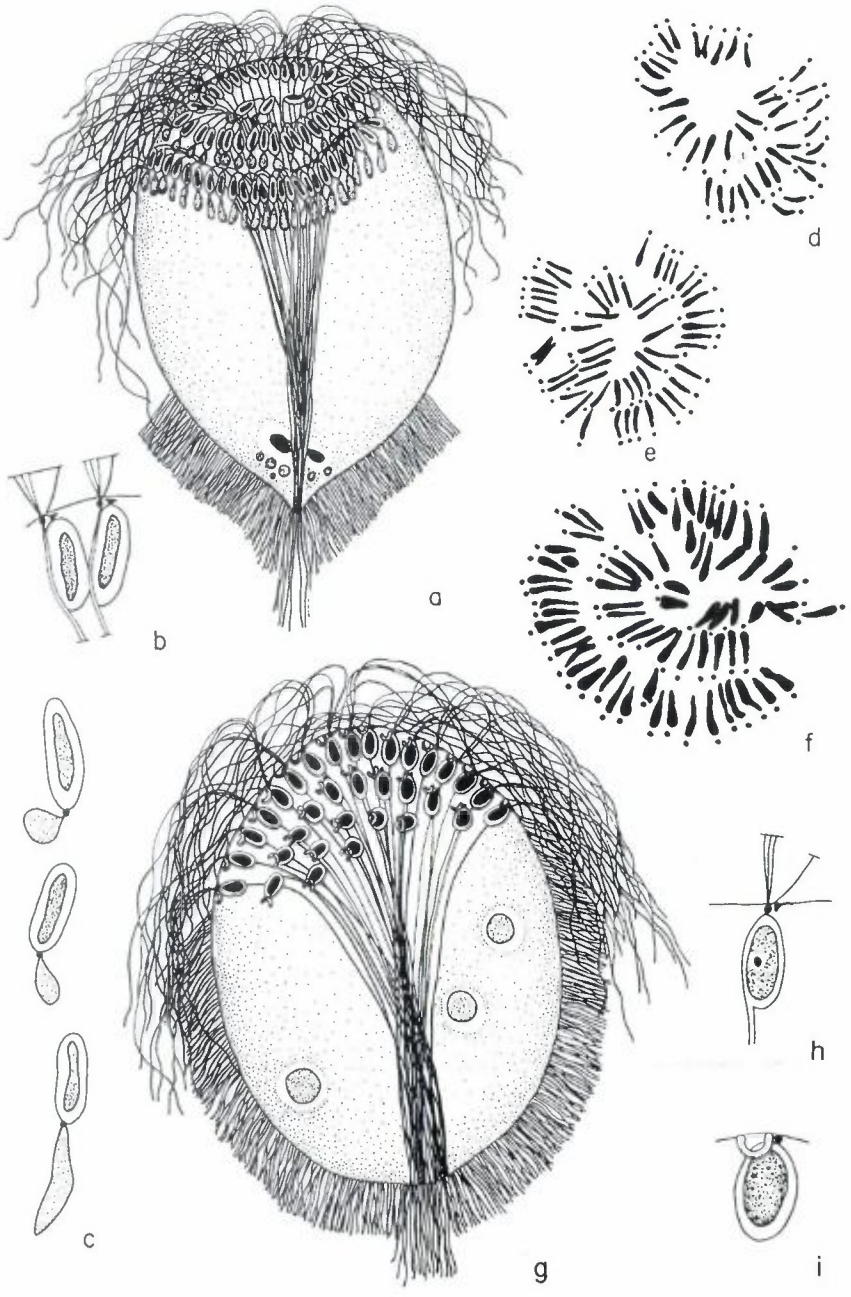


Plate I. *Stephanonympha silvestrii, S. grassii.*

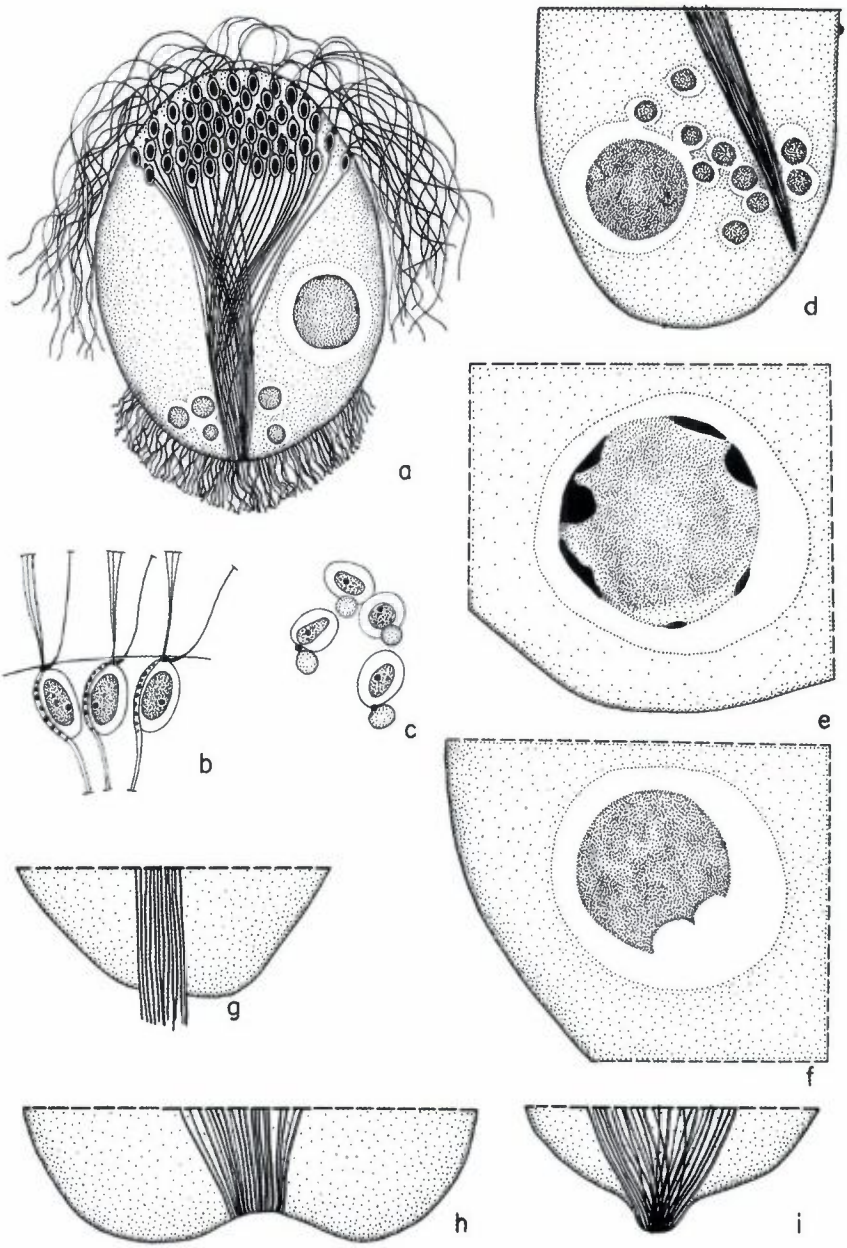


Plate II. *S. nelumbium.*

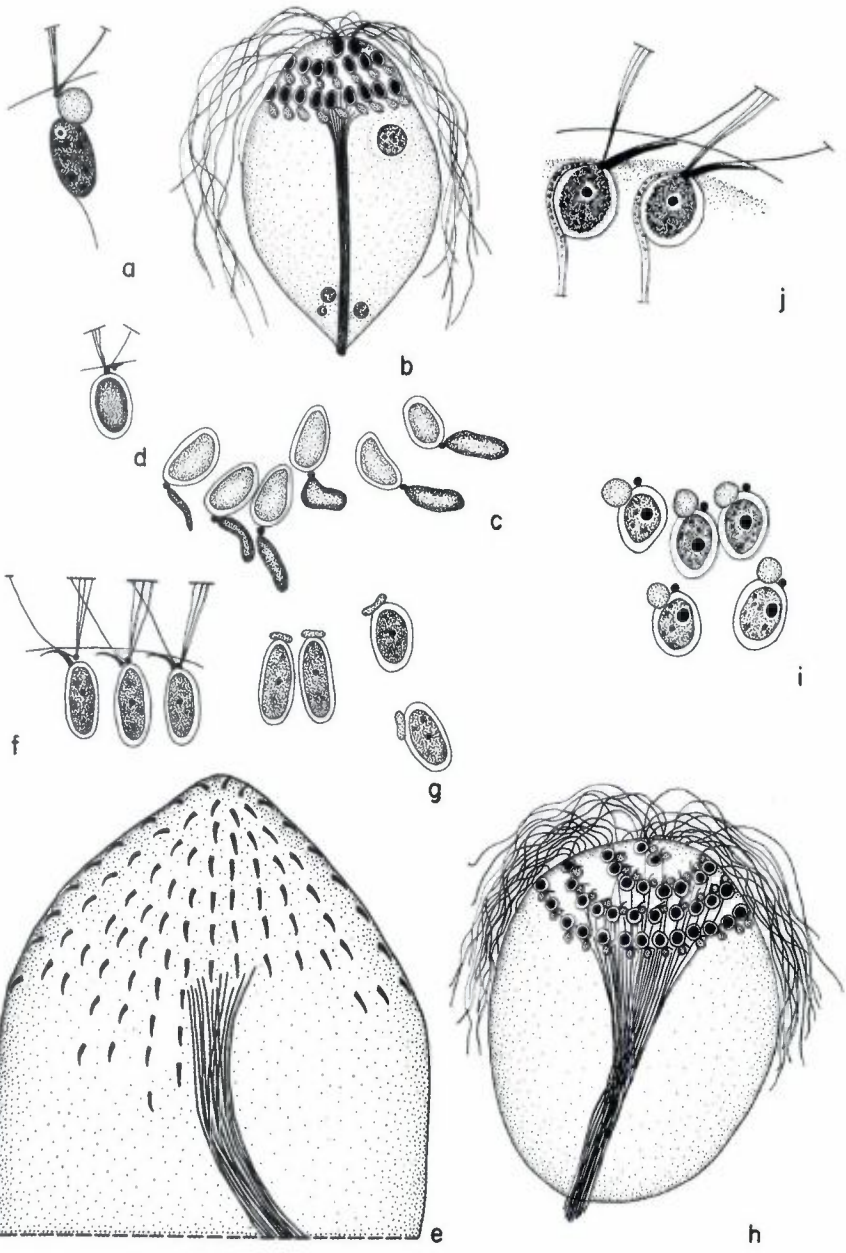


Plate III. *S. expleta, S. diversa, S. spinosa, S. rotunda.*

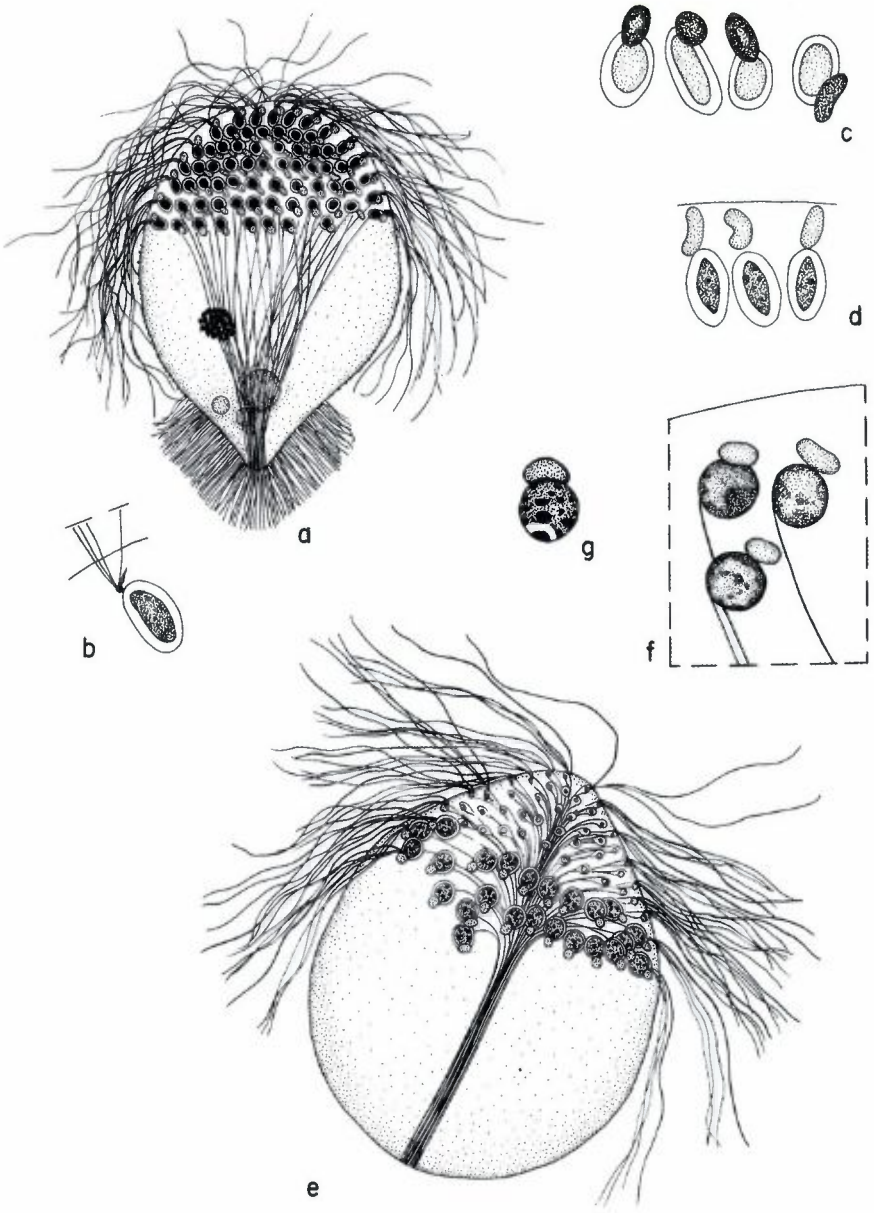


Plate IV. *S. rarita, S. assimilis, Calonympha grassii.*

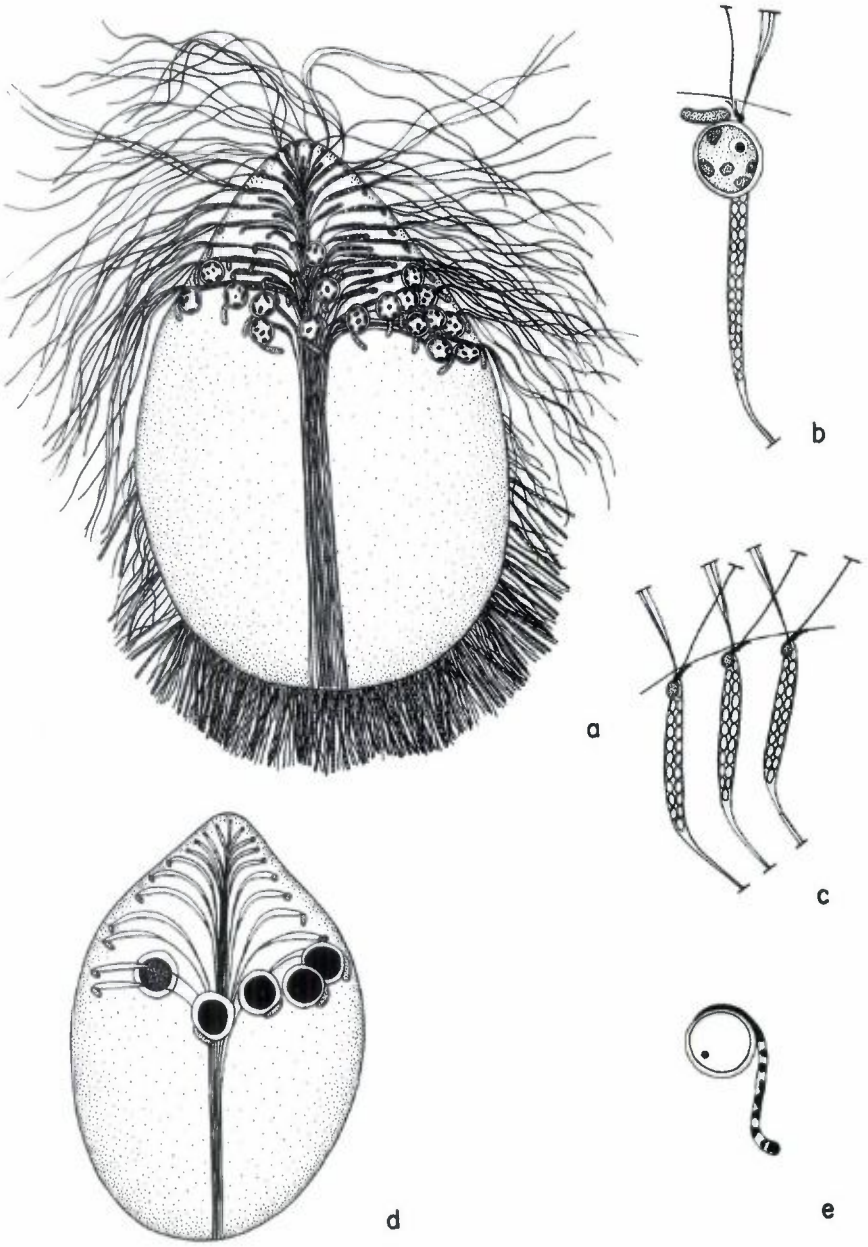


Plate V. *Calonympha angusta.*

Plate V

(a) *Calonympha angusta* from *Calcaritermes brevicollis*; (b) karyomastigont detail of *Calonympha angusta*; (c) akaryomastigont detail of *C. angusta*; (d) specimen showing number of nuclei in *C. angusta* from *Calcaritermes* sp. from El Salvador; (e) karyomastigont detail of *C. angusta*.

Plate VI

Some specimens showing nuclear division were found in all of the species of *Stephanonympha* we studied. Often it is possible to find dividing specimens; excellent material may be observed but rarely. The best examples of nuclear division seen by us were in *Stephanonympha grassii* and *Stephanonympha diversa*. When one slide was found with dividing specimens, there were usually many other specimens showing division on that slide.

When a specimen of *S. grassii* starts to undergo nuclear division the nuclei become irregularly dispersed (b). An individual nucleus may appear to be rounded, but very early the paradesmose is seen extending on either side, and on each side a kinetosome is observed (a). Sometimes a marked differentiation of the chromatin into four fairly distinct masses is observable. At this stage the body size of the mastigote is not noticeably increased. The bundle of axostyles is usually intact.

Soon the nucleus is elongated (c), and the four distinct chromatin areas become more widely separated (e). We did not see these clearly enough to be certain they are chromosomes (d). Next a slight pinching occurs in the middle of the elongated nucleus (e).

The nucleus next pulls apart and quickly forms two offspring nuclei (f). Soon the old axostyle bundle disperses and disappears (g). The appearance in the mastigote becomes that of a number of irregularly arranged nuclei, each with its new axostyle growing out (h).

Then the nuclei become organized at opposite ends of the cell, and the cell divides to form two mastigotes.

Plate VII

In *Stephanonympha diversa* we were able to study details of division of the individual nuclei. It was seen that in any early prophase the nuclear membrane stretches as the two new kinetosomes pull out at the ends of the paradesmose (a). In a number of instances the new undulipodia could be seen growing out from the kinetosome (b). The new undulipodium is very small and slender; as

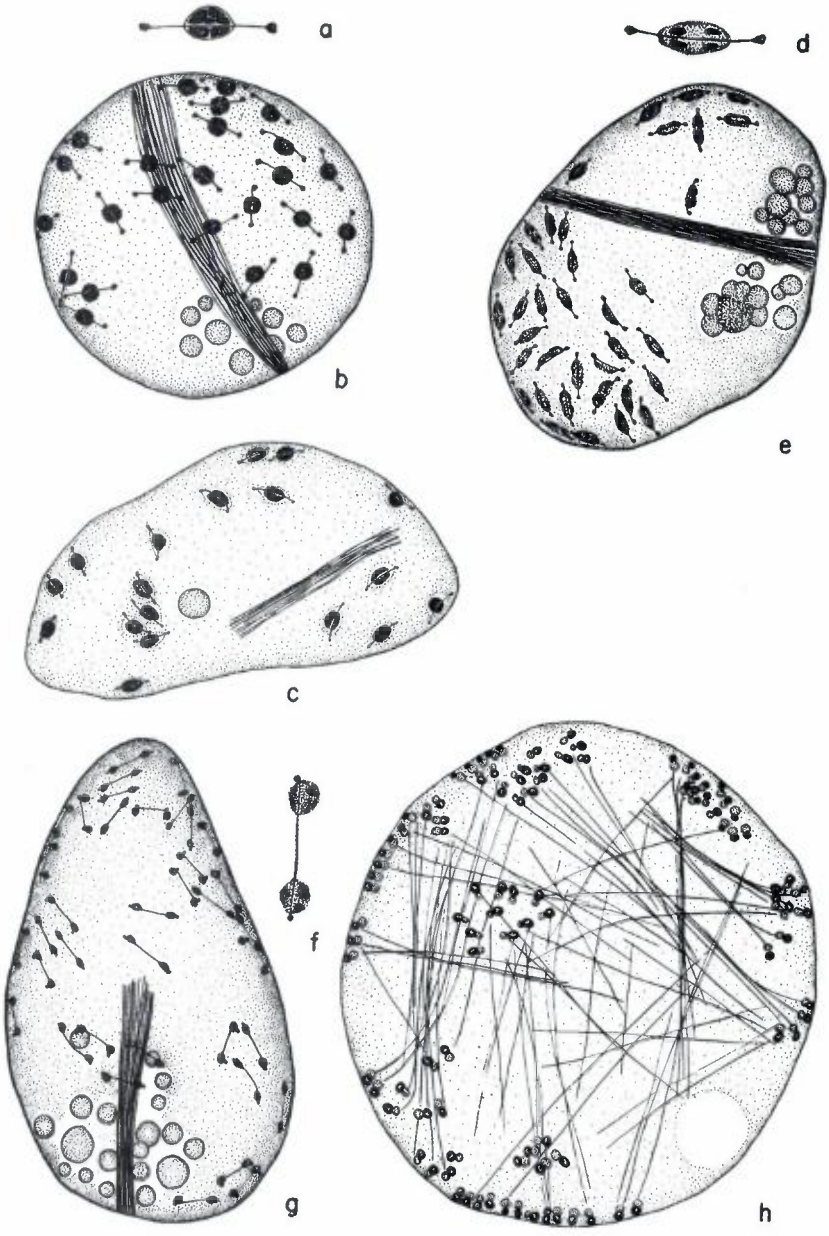


Plate VI. Nuclear division in *S. grassii*.

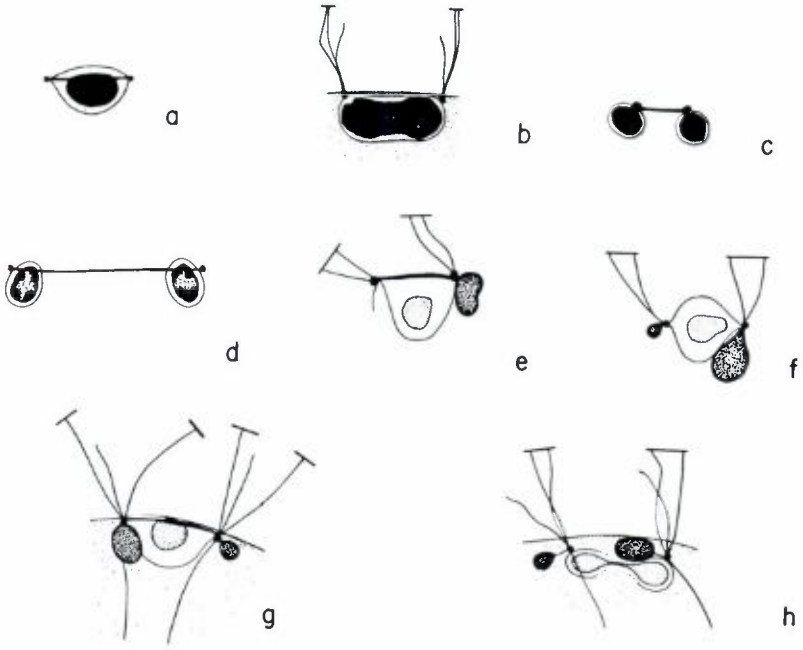


Plate VII. Nuclear division in *S. diversa*.

it increases in length it becomes stouter. Next the offspring nuclei are separate (c). Sometimes the chromatin matter is differentiated in the nuclei (d).

In silver-impregnated preparations we saw the development of the new parabasal bodies. Specimens were frequently observed with one normal parabasal body on one side and none on the other (e). A small new parabasal body is seen developing from the kinetosome that does not keep the old parabasal body (f). Often a parabasal filament is seen connecting both old and new parabasal bodies to their respective kinetosomes (h). The new parabasal body attains nearly normal size in early telophase (g).

[Description of Species]

Stephanonympha vescula, Stephanonympha spinosa, Calonympha

Calonympha grassii Foà

Calonympha grassii Foà, 1905, *R.C. Accad. Lincei*, (5), 14:542, text figs. 1 to 2.

Calonympha grassii Foà, Janicki, 1911, *Biol. Centralbl.*, 31:326, text fig. 6; Janicki, 1912, *Verh. Naturf. Ges. Basel*, 23:13, text figs. 7, 8; Janicki, 1915, *Z. Wiss. Zool.*, 112:620, pl. 15, figs. 30 to 35; pl. 16, figs. 36 to 40; pl. 17,

Table 1. Distribution of calonymphids in dry-wood-eating termites (kalotermitids).[1,2,3]

Family Calonymphidae, Grassi and Foà (1911)

Calcaritermes parvinotus
Calonympha sp.

Cryptotermes brevis
Calonympha grassii Foà
Snyderella bandeirantium
Stephanonympha sp.

Cryptotermes cavifrons
Snyderella sp.
Stephanonympha sp.

Cryptotermes domesticus
Stephanonympha nelumbium

Cryptotermes havilandi
Stephanonympha pyriformis
Stephanonympha silvestrii

Cryptotermes longicollis
Snyderella tabogae
Stephanonympha sp.

Cryptotermes sp. (India)
Stephanonympha reenstiernai

Glyptotermes parvulus
Diplonympha foae

Incisitermes banksi
Coronympha octonaria
Metacoronympha senta

Incisitermes emersoni
Coronympha octonaria

Incisitermes immigrans
Coronympha clevelandi
Stephanonympha sp.

Incisitermes marginipennis
Calonympha sp.

Incisitermes pacificus
Calonympha octonaria
Metacoronympha senta

Incisitermes platycephalus
Coronympha octonaria
Metacoronympha senta
Stephanonympha sp.

Incisitermes tabogae
Coronympha octonaria
Metacoronympha senta

Neotermes arthur-muelleri
Snyderella froilanoi

Neotermes bosei
Stephanonympha minuta

Neotermes chilensis
Stephanonympha calotermitis

Neotermes connexus
Stephanonympha silvestrii

Neotermes erythraeus
Stephanonympha silvestrii

Neotermes grandis
Calonympha avita

Neotermes hirtellus
Stephanonympha campinae

Neotermes insularis
Stephanonympha silvestrii

Neotermes sp. n. Sao Paolo, Brazil
Stephanonympha sp.

Neotermes tectonae
Calonympha patella

Rugitermes panamae
Calonympha grandis

Rugitermes rugosus
Snyderella ypiranga
Stephanonympha chagasi
Stephanonympha lindoya

[1] After Yamin, 1979, and Yamin addendum, 1981.
[2] No calonymphids reported from wood-feeding cockroaches or Mastotermitidae, Hodotermitidae, Termopsidae, Rhinotermitidae, or Serritermitidae.
[3] Prior to this work of Kirby, interrupted by his death in 1952. Table 2 lists those in the present paper.

Table 2. Distribution of calonymphids in kalotermitids: Additions to list in Table 1 based on this, Kirby's posthumous work.

Calcaritermes brevicollis
Calonympha angusta
Calonympha grandis

Calcaritermes emarginicollis
Calonympha angusta
Calonympha grandis

Calcaritermes nearcticus
Calonympha angusta
Calonympha grandis

Calcaritermes nigriceps
Calonympha angusta
Calonympha grandis

Calcaritermes parvinotus
Calonympha angusta

Calcaritermes sp. n. (El Salvador)
Calonympha angusta

Cryptotermes breviarticulatus
Stephanonympha grassii

Cryptotermes brevis (Hawaii, Galapagos, South Africa, Mexico)
Calonympha cryptotermitis
Calonympha grassii
Calonympha patella

Cryptotermes cavifrons
Stephanonympha rotunda

Cryptotermes cubicoceps
Calonympha angusta

Cryptotermes cynocephalus
Stephanonympha grassii

Cryptotermes domesticus
Stephanonympha nelumbium

Cryptotermes dudleyi
Stephanonympha nelumbium

Cryptotermes havilandi
Stephanonympha grassii

Cryptotermes kirbyi
Stephanonympha assimilis

Cryptotermes longicollis
Stephanonympha rotunda

Cryptotermes piceatus (Hawaii)
Calonympha grassii (Foà)
(= *C. avita*)

Cryptotermes queenslandis
Stephanonympha spinosa

Cryptotermes werwei
Stephanonympha grassii

Cryptotermes sp. (India)
Stephanonympha diversa

Glyptotermes angustus
Calonympha angusta

Glyptotermes caudomunitis
Stephanonympha grassii

Glyptotermes concavifrons
Stephanonympha vescula

Glyptotermes dilatatus
Stephanonympha grassii

Glyptotermes guianensis
Calonympha grandis

Glyptotermes kirbyi
Calonympha umbella
Stephanonympha vescula

Glyptotermes lighti
Stephanonympha vescula

Glyptotermes perparvus
Calonympha angusta
Calonympha grandis

Glyptotermes tuberculatus
Stephanonympha nelumbium

Glyptotermes sp. (Bandjar, Java)
Stephanonympha vescula

Glyptotermes sp. (Sumatra)
Calonympha sp.

Table 2. (Continued)

Kalotermes interioris	*Stephanonympha grassii*
Kalotermes isaloensis	*Stephanonympha grassii*
Neotermes castaneus	*Calonympha* sp. (Florida)
Neotermes connexus	*Stephanonympha silvestrii*
Neotermes dalbergiae	*Calonympha umbella* *Stephanonympha umbella* *Stephanonympha vescula*
Neotermes erythraeus	*Stephanonympha expleta* *Stephanonympha silvestrii*
Neotermes europae	*Stephanonympha expleta*
Neotermes grandis	*Calonympha avita*
Neotermes greeni	*Stephanonympha rarita*
Neotermes insularis	*Stephanonympha silvestrii*
Neotermes nudimalatus	*Stephanonympha expleta*
Neotermes sonneratiae	*Calonympha* sp. *Stephanonympha vescula*
Neotermes tectonae	*Calonympha patella* *Calonympha umbella* *Stephanonympha vescula*
Neotermes wervensis	*Stephanonympha grassii*
Neotermes zuluensis	*Stephanonympha expleta*
Neotermes sp. n. (Volcan de Santa Ana, El Salvador)	*Stephanonympha grassii*
Procryptotermes hamatus	*Stephanonympha expleta*
Procryptotermes jeannelanus	*Stephanonympha expleta*
Procryptotermes perezi	*Calonympha angusta*
Procryptotermes queenslandis	*Stephanonympha spinosa*
Proglyptotermes castaneiceps	*Stephanonympha penita*
Proglyptotermes longiceps	*Stephanonympha penita*
Proglyptotermes longus	*Calonympha angusta* *Stephanonympha penita*
Proneotermes perezi	*Calonympha grandis*
Rugitermes athertoni	*Calonympha grandis* *Calonympha angusta*
Rugitermes kirbyi	*Calonympha grandis* *Calonympha angusta*
Rugitermes magninotus	*Calonympha angusta*
Rugitermes panamae	*Calonympha grandis**
kalotermitid (unidentified, Puerto Rico)	*Calonympha grassii* *Calonympha irregularis*

* *Calonympha angusto* may be the same as *Calonympha grandis*. See Kirby's discussion, pages 41–49 here.

Table 3. Characteristics of calonymphids and criteria for their classification.

Calonymphid Characters[1]

Kinetosomes and undulipodia[1]

1. present
4. four free undulipodia: three forward and one trailing
13. recurrent undulipodium: "abnormal going up to the front of the cell"

Undulating membrane

18. present

Costa

25. costa, i.e., periodic fiber, present

Suprakinetosomal body

34. present

Axostyle–pelta complex

39. present with axostyle hypertwirled

Parabasal (Golgi) body

40. not V-shaped

Polymonad organization

41. permanent

Amoeboid cytoplasm

42. absent

Hydrogenosomes

43. present

Cryptopleuromitosis [extranuclear spindle microtubules fewer than 90° between two half spindles, nuclear membrane intact]*

44. present

Cysts

45. unknown

Classification[2]

KINGDOM PROTOCTISTA

Microorganisms and their larger descendants (exclusive of plants, animals, and fungi) composed of multiple heterologous genomes, products of symbiogenesis among bacteria.

Variations on mitosis and meiosis; many have undulipodiated[3] cells.

(Examples: algae, chytridiomycotes, slime molds, ciliates; includes an estimated 38 phyla and 250,000 species.)

Table 3. (Continued)

PHYLUM ZOOMASTIGINA

Heterotrophic mastigotes. Undulipodiated solitary cells and colonial forms; freshwater, marine; free-living, symbiotrophic, or necrotrophic.
(Examples of classes: amebomastigotes, opalinids, choanomastigotes, kinetoplastidans (e.g., trypanosomes), pseudociliates, diplomonads, pyrsonymphids, retortamonads.)

CLASS PARABASALIA

Single-celled, uni- and multinucleate protists with one or more parabasal bodies, with 3 to over 10,000 undulipodia.
(Examples: devescovinids, hypermastigotes.)

ORDER TRICHOMONADIDA

Symbiotrophs lacking peroxisomes and mitochondria; characteristic kinetids, see Fig. 1.b.
(Examples: *Dientamoeba, Trichomitus, Hexamastix, Trichomonas, Monocercomonas, Mixotricha.*)

FAMILY CALONYMPHIDAE

More than two karyomastigonts.
(Six genera; see Table 4.)

* Same as "closed extranuclear pleuromitosis" or simply "pleuromitosis" in which the spindle, both central and chromosomal fibers, are entirely extranuclear. Ribbon-shaped derivatives, called "atractophores," form from the kinetosomes. Sometimes atractophores have amorphous atractospheres (=centrospheres) at their ends. The central spindle is a narrow bundle of microtubules (=paradesmose) that connects the atractophores. See Raikov (1982) for details.

[1] From Viscogliosi et al. (1993). Numbers (1)–(45) refer to their Table 2 (page 27) and Appendix 1 (page 421). Missing numbers are characters in other trichonymphids irrelevant to calonymphids.

[2] Based on *Handbook of Protoctista* and *Illustrated Glossary of Protoctista* (Margulis et al., 1990; 1993).

[3] [9(2)+2] structure underlain by a [9(3)+0] kinetosome (see below page 59).

Table 4. Calonymphid genera: Summary of calonymphid classification.

KINGDOM	Protoctista
PHYLUM	Zoomastigina
CLASS	Parabasalia (Hollande)
ORDER	Trichomonadida* (or Polymonadida)
FAMILY	Calonymphidae (Grassi and Foà)
GENERA	*Calonympha* (Foà)
	Coronympha (Kirby)
	Diplonympha (Grassi)
	Metacoronympha (Kirby)
	Snyderella (Kirby)
	Stephanonympha (Janicki)

* Preferred by Kirby as explained on pages 12 to 16 here. See Margulis et al., (1990, 1993) for discussion of these higher taxa.

Table 5. Kirby's *Calonympha* and *Stephanonympha* species.

Calonympha	*Stephanonympha*
angusta	*assimilis*
avita (=C. grassii)	*calotermitis*
cryptotermitis	*campinae*
grandis	*chagasi*
grassii	*diversa*
irregularis	*expleta*
octonaria	*grassii*
umbella (= C. patella?)	*lindoya*
	minuta
	nelumbium
	penita
	pyriformis
	rarita
	reenstiernai
	rotunda
	silvestrii
	spinosa
	umbella
	vescula

figs. 42 to 44; Grassi, 1917, *Mem. Accad. Lincei*, (5) 12:390, pl. 10, figs. 42 to 43; Calkins, 1936, *Puerto Rico J. Pub. Health Trop. Med.*, 12:173, text fig. 2.

Calonympha grassii Pascher, 1929, *Arch. Protistenk.*, 68:290.

Calonympha cryptotermitis Calkins, 1936, *Puerto Rico J. Pub. Health Trop. Med.*, 12:173, text fig. 3, pl. 1, figs. 12 to 14.

Calonympha irregularis Calkins, 1936, *Puerto Rico J. Pub. Health Trop. Med.*, 12:174, text fig. 4, p. 1, figs. 15, 15a.

Calonympha avita Brown, 1941, *J. Tenn. Acad. Sci.*, 16:357.
Type host – *Cryptotermes brevis* (Walker).

T-320. Silvestri, "*Calotermes (Cryptotermes) grassii* Silvestri, MS." Iquique, Chile.
T-261. Colima, Mexico.
T-240. Puerto Rico.
T-555. St. Augustine, Trinidad.
T-321. "*Cryptotermes piceatus* Snyder." Hawaiian Islands.
T-547. Chilata, El Salvador.
T-4011. Durban, South Africa.
T-4600. Lima, Peru.
T-238. "*Cryptotermes darwini* Light." Galapagos Islands.
T-253. (Larsen 18.) "*Cryptotermes darwini* Light." Galapagos Islands.

Calonympha grassii was described by Anna Foà (1905) from a then unnamed termite sent from Iquique, Chile, by Professor DeVescovi. When Janicki (1911, 1915) published a description of the same mastigote from the same source, he designated the termite by the name *Calotermes (Cryptotermes) grassii*. That name had evidently been supplied by Silvestri, but the species was never published. A few years ago Professor Silvestri sent to us a drawing of a soldier. Professor Emerson wrote in January 1950 that he was placing *C. grassii* Silvestri in synonomy with *C. brevis*, though doubtfully in the absence of specimens. After later examination of the drawing, he wrote that it was in conformity with *C. brevis* and that he was convinced that the two species were the same. When the conformity in the protists is also considered, it seems practically certain that the type host of *Calonympha grassii* is actually *Cryptotermes brevis*.

Janicki (1911, 1912) dealt with some features of this mastigote and finally (1915) published an excellent description. At the present time published information about the genus consists almost wholly of Janicki's fine work on this species. Many references have been made to it in the literature, without addition of new material, and several other species have been named.

Calkins (1936) described briefly a number of mastigotes from termites of Puerto Rico, and among them recorded three species of *Calonympha*. *C. grassii* Foa was recorded from an unidentified termite obtained from a pine box, and a new species, *Calonympha irregularis*, was described from the same colony of termites. A second new species, *Calonympha cryptotermitis*, was recorded from *Cryptotermes brevis*, obtained from a window frame and identified by Dr. Snyder. Both termites contained also *Devescovina*, that from the window-frame termite being *D. striata* Foà (*D. paralemniscata* Calkins is a synonym).

The habitat and faunules of these termites indicate that both were *Cryptotermes*, and it is probable that both were *Cryptotermes brevis*. The new species of Calkins are synonyms of *Calonympha grassii*, according to Brown (1941a). His explanation of this opinion appears in his manuscript doctoral thesis filed in the University of California library.

Brown (1941a) named a new species, *Calonympha avita*, found in *Cryptotermes piceatus* of Hawaii. In a review of the genus *Cryptotermes*, Emerson concluded that *C. piceatus* is a synonym of *C. brevis*, informing us of this in a letter in January 1950. The faunules are in conformity with that conclusion, and *Calonympha avita* is considered in this monograph to be a synonym of *Calonympha grassii*.

Cryptotermes brevis is a widespread termite, and we have had preparations of the protists from hosts in Mexico, El Salvador, Puerto Rico, Peru, the Galapagos Islands, and South Africa. We have been able to study a slide of *Calonympha grassii* from the type colony sent by Professor Silvestri and bearing a label showing it was from Iquique, Chile. Numerous specimens in an excellent state of preservation are present on the slide.

The lengths and widths of the mastigotes in *Cryptotermes brevis* were as follows: Chile colony (our measurements) 58 (45 to 72) μm by 42 (35 to 53) μm; Mexico colony 55 (33 to 84) μm by 39 (24 to 56) μm; Hawaiian colony (Brown, 1941a) 49 (26 to 81) μm by 39 (18 to 41) μm; Peru colony 41 (23 to 78) μm by 31 (16 to 46) μm; Puerto Rico colony 45 (28 to 78) μm by 33 (24 to 57) μm. Most of the measurements are based on a sampling of 25 specimens from several slides. It will be noted that the lengths do not reach the record by Foà (1905) of a range from 40 to 90 μm, and an average of 80 μm, in the Chile termites, but she presumably had living material, and in the same material Janicki (1915) recorded a maximum length of 69 μm, close to that we found; all the measurements fall far short of the lengths reported by Calkins for *Calonympha irregularis*, 80 to 148 μm, though his widths are well within our ranges. His material was probably distorted in smearing, as evidenced by his statements that the length was from two to six times the width (a proportion never normal for *Calonympha*), that the axial strand terminates at some distance from the posterior end (which it almost always meets in normal specimens), and that the nuclei are distributed throughout the endoplasm (here surely irregularity is due to mechanical damage).

The number of nuclei, and hence the number of karyomastigonts, ranges from 8 to 29 in Brown's *Calonympha avita* (Brown, 1941a). The range we found in the various other hosts was from 9 to 68, the average number ranging in different individual hosts from 23 to 41. The average was 41 and the range 16 to 68 specimens of *Calonympha grassii* from *Cryptotermes brevis* of Chile.

Nuclei were counted in nearly one hundred specimens of *Cryptotermes brevis* from Mexico, and the range found was from 15 to 46 with an average of about 30. In the Puerto Rico material the number found did not exceed 40, and there were more specimens with fewer than 20 than in the Mexican material, bringing the average to 23.

There is essential agreement in the numbers in different hosts, but some individual slides may have predominantly specimens that have smaller numbers. Such material evidently led Calkins to the opinion that *Calonympha cryptotermitis* was a different species. He observed it as the only *Calonympha* in certain preparations, and found only 12 to 16 karyomastigonts; it is most probable that if more slides had been examined, a greater range would have been found.

The karyomastigonts are not perfectly regular in arrangement. In specimens with a small number there is often a single ring, complete or broken, and one end may often be displaced to a more posterior level than the other. Thus there seems to be some tendency toward a spiral. There are often two rows of nuclei around part of the body or completely, and in some larger forms there are in part three rows. Occasionally there is a greater accumulation of nuclei in one region than in another, so that there are on one side as many as 5 or 6 layers as Janicki (1915) reported, but that is exceptional. The nuclei are always peripheral in position, with a constant relationship to the kinetosomes and undulipodia. They are always posterior to the akaryomastigonts, never mingled with them.

The arrangement of the akaryomastigonts shows a high degree of regularity, and their spacing is rather even. The outer boundary of the akaryomastigont zone, which is the periphery of the anterior part of the body, has the form of a bowl. In some specimens the contour of the bowl tends to be an arc of a circle, but more usually its sides are steeper, and the form is somewhat conical with a rounded apex. Janicki (1915) stated that in *Calonympha grassii* the number of akaryomastigonts is much greater than that of the [karyomastigonts with] nuclei.

Brown (1941a) made counts in 15 specimens of *Calonympha grassii* and found that 39% were karyomastigonts. One feature by which he differentiated *Calonympha avita* (= *C. grassii*) in *Cryptotermes piceatus* (= *C. brevis*) was the supposed greater proportion of karyomastigonts. In 20 specimens he recorded the number of karyomastigonts as 19 to 55 and that of akaryomastigonts as 4 to 37, averaging 34 and 22 respectively, with a ratio of 61% karyomastigonts. If it were true that in one form of *Calonympha* the karyomastigonts consistently outnumbered the akaryomastigonts by almost two to one, whereas in another form the ratio was consistently the reverse, there

would be a justification for specific distinction. However, examination of our preparations has convinced us that the ratios show no such distinction and that there is a wide range of variation in the relative proportions of the two types of mastigonts.

The specimens in certain hosts tend to be larger than in others and may have a greater number both of akaryomastigonts and karyomastigonts. In the material of *Cryptotermes brevis* from Mexico, with 15 to 46 nuclei, the akaryomastigonts amounted on the average to about double this number. In smaller specimens the proportion might be lower than that. In other hosts a greater number of smaller specimens of *Calonympha* occurred, and in some of these the akaryomastigonts might be as few as 10 or fewer, with the number of karyomastigonts somewhat greater. A proportionately large number of smaller specimens occurred in *Cryptotermes brevis* of Durban and in *Cryptotermes brevis* of Hawaii ("*Cryptotermes piceatus*"). In the specimens from the latter host, estimates of the number of mastigonts gave a variation from more than twice as many akaryomastigonts as karyomastigonts to fewer of the former than the latter. The average in the rough estimates was 28% of the former and 22% of the latter. It is exceptional for the nucleated mastigonts to outnumber the others, though some such specimens occur.

The four undulipodia leaving the prominent peripheral kinetosome of each mastigont are differentiated into a bundle of three and a separate fourth. Foà showed only one undulipodium on each mastigont of *Calonympha grassii*. Janicki (1911, 1915) stated that there are four undulipodia, although his figures of the mastigotes do not show four, but only one, two, or three in a mastigont. He stated in both papers that one undulipodium is thicker than the others. Our protargol preparations showed the separate undulipodium to be appreciably thicker, though not greatly so, but it could not be characterized as almost bandlike, as was stated by Janicki (1915). Certain observations suggest that Janicki's statement about the large difference in thickness among undulipodia was due to his observing the bundles of undulipodia on one hand and the individual undulipodia on the other. The undulipodia often adhere in bundles for some distance from the surface of the body, and these bundles appear much thicker, of course, than the individual undulipodia.

Brown (1941b) reported the presence of a cresta in a member of the genus *Calonympha*. In his manuscript a minute cresta along the base of the separate undulipodium is described and figured in *Calonympha avita* (= *Calonympha grassii*) from *Cryptotermes brevis* ("*C. piceatus*") of Hawaii and *Calonympha patella*. In *Calonympha grassii* from *Crypotermes brevis* of Chile, Brown reported no cresta, and in that from material examined from

hosts of Puerto Rico, Mexico, and Peru, he observed a second, elongated granule in the position of and comparable to the cresta. These were the first reported observations on the presence of a cresta in this genus.

Observation of the minute cresta is possible only in material favorably stained with iron hematoxylin. In *Calonympha grassii* it is so small that it is seldom possible to make it out as a distinct organelle. However, there is no doubt that it is present in the species. It has been recognized by Brown and by us in mastigotes from several colonies of *Cryptotermes brevis*, including specimens from the Hawaiian Islands, Galapagos Islands, South Africa, and Mexico. It is seldom possible to determine the outline of the cresta. Whenever it is seen clearly, it appears as a straight or somewhat curved rod, thicker at the end where it meets the kinetosome; often it appears merely as a thickened region along the base of the separate undulipodium. Its length is about 0.5 μm.

The bundle of axostyles is usually quite compact, appearing as a longitudinally striated compact rod, from which in many specimens some individual axostyles spread out separately from the sides. These are all gathered together in the posterior part, which is more or less truncate and usually either meets the posterior end of the body or projects a short distance beyond it. In some specimens there is an indentation where the axostyle bundle meets the posterior end of the body. In many specimens, probably in those where the contour of the body has not been well preserved, the bundle of axostyles is curved in the cytosome. The axostyles do not begin to splay out usually until about the level where the akaryomastigonts spread out in a symmetrical form, at necessarily more anterior levels, like the petioles of a bowl-shaped, or rounded, conical inflorescence.

In their anterior parts the axostyles of the akaryomastigonts show a relatively great expansion into a clavate structure, in marked contrast to the filamentous form of their more posterior part. Foà (1905) showed this structure plainly and described it as a small ampoule or calyx of thickened protoplasm, along an edge of which runs a filament that continues toward the interior of the mastigote. Janicki (1915) described the enlargement as a region of dense homogeneous plasma in the spindle form bordering the axial filament on one side and enclosing the parabasal body. Brown (1941a) pointed out that this differentiation is actually the expanded capitulum of the axostyle, and we are in agreement with his opinion.

The capitula of the karyomastigont axostyles are expanded, and along one edge is a row of iron hematoxylin-staining granules, which appear to be fused in a bar in some mastigonts. These granules may be present in varied number or may not show at all, depending upon the material and technique. Because of the presence of the nuclei, it is difficult to observe the characteristics of the

capitula of the karyomastigonts. The clavate capitula of the akaryomastigonts are rounded in cross section, the more posterior ones having a diameter of 2 μm. Those of the apical akaryomastigonts are smaller. The series of small granules shows also along the edge of many of the akaryomastigont capitula. There may be a number of isolated granules, or a row, of a series apparently fused into a bar. The presence of these structures possibly influenced Foà and Janicki in their opinion that the axostyle filament borders the fusiform differentiation.

A small rounded parabasal body was first described and figured by Janicki (1911). Janicki (1915) described this in more detail as a very simple and poorly developed parabasal apparatus, consisting of rounded or oval, less often longitudinally elongated, corpuscles, along one edge of which a black line could be seen, indicating a possible parabasal filament. The relationship of the apparent filament to the kinetosome could not be determined.

The parabasal bodies of the karyomastigonts have more or less the form of thick discs, as Brown (1941a) described them in *Calonympha avita.* When not adequately stained, they are likely to be interpreted as spherical. It is clear that a difference in description, of the shape as spherical or disc-like is not significant, and there may actually be variation within a species in these two forms. In some aspects the parabasals appear as circular bodies lying close to the nucleus, in others they appear as stout rods; and light microscopy does not permit determination of the exact form of an individual body. The regular occurrence of these two forms seems to indicate that the differences in shape are due to observations of the same body in different views. Their diameter is 1.5 to 2 μm, and the thickness about 1 to 1.5 μm. Some parabasals are concave on the surface where they are closely applied to the nuclear membrane; others seem to have flat surfaces that touch the nucleus. The parabasals of the akaryomastigonts are smaller and appear rounded or somewhat elongated. The dimensions of those of the more posterior akaryomastigonts are 1 to 1.5 μm, and those of the more anterior akaryomastigonts are smaller, grading down to granule-like bodies not more than 0.5 μm in diameter. We have not seen a filament alongside of the parabasal body, which Janicki reported with some uncertainty.

The nuclei are spherical or nearly spherical and have a diameter of about 2 to 2.5 μm. Janicki (1915) remarked on the difference between their round or oval shape and the marked elongation in one diameter in *Stephanonympha.* Certain larger granules retain iron-hematoxylin stain much more tenaciously than do the greater number of smaller granules filling the interior. A single layer of peripheral granules set off in a clear zone is generally present. This corresponds to the peripheral nucleolus of the typical trichomonad nucleus.

There may also be several other deep-staining granules, which, however, are not similarly set off in a clear area.

Janicki (1915) observed in the mastigotes he studied that the body posterior to the mastigont region contains numerous bacteria and particularly wood fragments of various sizes and that occasionally in the part near the nuclear zone there is an aggregation of plasma, somewhat oval in outline and intensely stainable with iron hematoxylin, in which bacteria apparently predominate. The aggregation suggested to him the postnuclear zone around the axostyle of *Joenia* in which bacteria are collected, though he called attention to the fact that it is not constant in *Calonympha*, whereas it is characteristic of *Joenia*.

The cytoplasm posterior to the mastigonts usually contains abundant wood particles, which are most often evenly distributed throughout the region. In some specimens there are certain peripheral regions in which the wood is less abundant or absent, but there is no evidence of a regularly occurring inclusion-free ectoplasm. On the slide from Chile, the hematoxylin stain was poor, but a large number of granules, probably corresponding to those Janicki mentioned as bacteria, were stained. An abundance of wood particles could be seen, especially by phase-contrast observation. Our other material also showed wood and granules or short rods stainable by iron hematoxylin.

Spherules, like those so frequent in species of *Stephanonympha*, were absent from *Calonympha grassii* in most preparations. On the series of slides from *Cryptotermes brevis* collected from El Salvador, however, conspicuous cytoplasmic spherules were present in almost all specimens. Often there was an aggregate of several spherules of small size around the posterior part of the axostyle. Besides there were usually several larger spherules of varied size, each surrounded by a clear zone, in various parts of the cytoplasm. The largest ones, as they occur in a few of the mastigotes, reach a diameter of 10 μm. Some show a vacuolated interior, often a large vacuole seemingly open at one side, as in certain large spherules of *Stephanonympha*. In mastigotes on some slides in the series the number of spherules is smaller than in the mastigotes on others. The spherules appear brown in alum hematoxylin-stained material, and they impregnate rather deeply when the protargol technique is used.

A striking feature of protargol preparations is the presence in all specimens of abundant, conspicuous, deeply impregnated bodies in the cytoplasm. They have been studied in mastigotes from *Cryptotermes brevis* from the localities other than Chile, Peru, and Hawaii; from these three localities no silver preparations were available. The bodies are distributed throughout the post-mastigont cytoplasm, and some extend even into the mastigont zone. In many specimens the bodies are short stout rods reaching about a micron or so in length and having rounded ends. The longer rods often appear double, or

there are paired or isolated granules. Some of the bodies in some specimens, and most of those in others, are extended into filaments drawn out at one or both ends.

Small cytoplasmic bodies are often revealed in preparations stained with iron hematoxylin, and less distinctly in some stained with alum hematoxylin. They may be rods or granules, but they seem much smaller than the silver-impregnated bodies and do not show such definitive characteristics of shape. It seems probable that some of the bodies stained by hematoxylin techniques correspond to the silver-impregnated bodies, and that others may not. The forms of the latter conspicuous bodies suggest division, but it is not probable that they are bacteria. They may be chondriosomes [mitochondria]. [Although "chondriosome" is an old name for mitochondria and is likely what Kirby meant, Miklos Muller notes that it is even more probable that the bodies are hydrogenosomes. Personal communication, 1993.]

The aggregate stainable plasma containing bacteria that Janicki described in some specimens was not seen in any of our material.

Foà (1905) observed and illustrated a quantity of filiform spirilla, adherent to the whole surface of the body posterior to the mastigont zone; and Janicki (1915) saw indications of these on his stained slides. On the slide from Chile in our possession, spirochetes are widely distributed on the bodies of most of the mastigotes, though not in the dense tufts characteristic of some other Calonymphidae. They are also present in *Calonympha grassii* in other hosts, but there is variation in their presence and distribution in different individuals. Often they are widely and rather sparsely distributed on the whole postmastigont part of the body. Frequently also they are mainly restricted to the posterior end, and sometimes there is a tuft at the region of the posterior end of the bundle of axostyles. On some slides, and on some individuals from other slides, they are absent.

There is no peripheral differentiation in the characteristics of the cytoplasm up to the cell boundary. It contains a mass of granules and wood fragments, extending fully to the membrane, or leaving only a narrow zone of cytoplasm free of the inclusions. The boundary appears as a definite membrane. Some slides were studied in which apparently the specimens had been damaged in preparation; that was true also of *Devescovina* on the same slides. In many specimens the inclusions in the postmastigont region are more concentrated in a mass, peripheral to which there is a clear zone bounded by a sharply defined membrane. The membrane continues around the mastigont region where there has been no cytoplasmic alteration. Often outside of the body membrane there is a more or less irregular but rather well-defined membrane. This zone is extended only around the postmastigont body. It varies greatly in extent,

from a narrow zone around part of the posterior region of the body to a very broad clear region around the whole postmastigont region, and sometimes including part of the mastigont region. On some other slides specimens do not show the outer clear zone, but the cytoplasmic inclusions are concentrated and surrounded by a clear zone bounded by a very definite appearing wall. These appearances suggest, superficially, encysting or encysted organisms. However, the apparent wall never extends completely around the body, the mastigont region being unaltered or only partly altered. There is no tendency for the cells to be rounded or surrounded by a separated cyst wall. They result from alteration in the fluid content of the cytoplasm and the exudation of fluid to form the outer clear zone. We find no reason for regarding these as cysts or forming cysts; instead they are abnormalities resulting from improper preparation.

Janicki (1915) described a fairly complete series of division stages. There is division of both akaryomastigonts and mastigonts, with a paradesmose in both types; according to him the paradesmose becomes the new axial filaments of the offspring mastigonts (an erroneous opinion). The old parabasal body is retained at one pole, and a new one develops at the other. The mastigonts organize into two groups, and fission takes place.

The iron hematoxylin-stained slide from the Iquique termite we studied showed numerous prophase nuclei, but no later division stages. Most specimens were overdestained, though there were some thicker areas of the film where the stain was relatively heavy. The interphase nucleus is ellipsoidal or spherical and measures about 3 to 4 μm in diameter. The chromatin material fills the interior. The greater part of this is finely granular and is pale in well-destained nuclei, appearing light gray. At the periphery, in clear zones, there are more deeply staining granules. As nuclei advance in the prophase they become somewhat enlarged, and the whole stainable contents become aggregated at the membrane. This takes the form of five stout bands, arranged all in the same direction and extending from end to end of the nucleus. In optical section it can be clearly seen that there are five of these bands, evenly spaced around the nucleus. We have not seen the earlier stage of a continuous convoluted filament, which Janicki, somewhat doubtfully, represented. The chromosomes seem to be separated from the beginning. They stain intensely with iron hematoxylin, in contrast to the pale stain taken by the chromatin material of interphase nuclei in the same areas of a slide.

According to the accounts by Foà and Janicki, spirochetes adhere to the whole area of the body posterior to the undulipodia. However, on the slide we examined there was no indication of the dense aggregation of spirochetes that often occurs on many mastigotes of termites.

***Calonympha angusta* sp. nov. (no plates)**

Calonympha grandis Brown *nomen nudum*, 1941, *J. Tenn. Acad. Sci.*, 16(4):358.

Type host – *Rugitermes panamae* (Snyder). Panama.
T-191. Barro Colorado.
T-120. Barro Colorado.

Additional hosts –

Calcaritermes brevicollis (Banks). Panama.
T-125. Barro Colorado.
T-130. Barro Colorado.
T-197. Barro Colorado.
T-206. Barro Colorado.
T-233. Taboga Island.

Calcaritermes emarginicollis (Snyder). Costa Rica.
T-158. Estrella.

Calcaritermes nearcticus (Snyder). Florida.
T-535. Ortega.

Calcaritermes nigriceps (Emerson). British Guiana.
T-673 (Emerson). Kartabo.

Calcaritermes parvinotus (Light). Mexico.
T-265 (Light 183). Colima.

Calcaritermes sp. El Salvador.
T-545. Volcan de Santa Ana.
T-548. Chilata, Dept. Sonsanate.

Cryptotermes cubicoceps (Emerson). British Guiana.
T-674 (Emerson). Kartabo.

Glyptotermes angustus (Snyder). Panama.
T-127. Barro Colorado.

Glyptotermes guianensis (Emerson). British Guiana.
T-675 (Emerson). Kartabo.

Glyptotermes perparvus (Emerson). British Guiana.
T-676 (Emerson). Kartabo.

Rugitermes athertoni (Light). Ecuador.
T-4612 (Von Hagen 28). Hac de Tenguel.

Rugitermes kirbyi (Synder). Costa Rica.
T-135. Cartago.
T-148. Cartago.

Rugitermes magninotus (Emerson). British Guiana.
T-678 (Emerson). Kartabo.

Proneotermes perezi Holmgren. Central America.
T-141. Cartago, Costa Rica.
T-544. Mt. Cacaguatigue, El Salvador.

The name *Calonympha grandis* was published by Brown (1941b) and mentioned in the list of three new species by the words "*C. grandis* (from *Rugitermes panamae*)." No other statement about it appears in print, so the name must be regarded as a *nomen nudum*. An illustrated description exists in Brown's manuscript thesis filed in the University of California library. He found a range in length from 49 to 140 μm, with a mean of 82, and in width from 34 to 83 μm, with an average of 53. The nuclei ranged in number from 17 to 52, with a mean of 34. Brown (1941a) reported it to be characteristic of this mastigote that 34% of the mastigonts are karyomastigonts. The nuclei are relatively large in size, ranging from 4.1 to 6 μm in diameter. He found the parabasal bodies to be usually in the form of concave discs ranging from about 1.8 to 3.5 μm in diameter and 0.9 to 1.3 μm in thickness, those of the karyomastigonts being the larger ones and not so closely juxtaposed to the nuclei. The capitula of the axostyles are clavate, usually large, and are provided with granules. The bundle of axostyles is compact. No cresta was seen. Adherent spirochetes are abundant.

Calonympha angusta is here recorded as the only species of *Calonympha* in 16 termite hosts belonging to five genera. The most unexpected of these records is that from *Cryptotermes cubicoceps*. This termite from British Guiana has, except for the absence of *Trichonympha*, a faunule like those of *Rugitermes panamae* and *Rugitermes kirbyi*. *Calonympha angusta* is the species of that mastigote genus in all of the species of *Calcaritermes* that have been examined and in all of those of *Rugitermes*, but it has not been found in all the species of *Glyptotermes* in this region, and it has been found in no *Glyptotermes* species in any other part of the world. Its hosts have in common, occurrence in middle America only, but they are taxonomically varied, and three genera of the hosts have species in other parts of the world that do not have the mastigote. It appears that it has become adapted to various kalotermitids because of their geographic proximity and is not distributed according to phylogenetic relationships in strict host isolation. But that explanation may be superficially attractive rather than sound; it will not be dwelt on here, as a full discussion requires a detailed consideration of the whole faunules of all the termites of these regions and of the state of taxonomy of the hosts.

We are not sure of our position, either, in concluding that all of the members of the genus *Calonympha* in this list of hosts belong to the single species. The main reason for the conclusion has been the impossibility of giving any sets of

characteristics that would differentiate populations. There is great variation in the number of mastigonts, the size of nuclei, and the size and shape of the parabasal bodies of the karyomastigonts. Specimens of the mastigotes could be selected between which definable specific characteristics could be determined. In some instances it would appear that almost the whole population in a host consisted of one form and that of another host of another. But when the characteristics of *Calonympha* on many slides from many colonies of the 16 hosts are surveyed, the gradation is found to be complete; there are many different combinations of the characteristics that seem to be usable, and each shows differences in populations where taxonomic distinctions could be made. Thus it has been decided to keep the whole complex under one specific name, though recognizing that it is a nominal rather than a real entity. Of course, that is true of many of these organisms that evidently have no sexual reproduction and have been isolated in different hosts for very long periods of time.

The body is elongated in form and has a length which at one extreme is greater than the width by about half, and at the other about equals the width. As a general average, the width is about 80% of the length. The posterior end of the body is broadly rounded and is deformed easily in the process of preparing the smears. The anterior part retains its form better, apparently because of the presence of the mastigonts. It is often more or less narrowed and broadly conical with a rounded apex, but it varies from having a broadly convex contour to a narrowed one.

In the greater number of specimens the mastigont zone occupies about a third of the length of the body; it may be as much as half or as little as a quarter.

Measurements were made of 25 specimens from each of the hosts, except for *Calcaritermes parvinotus* and *Cryptotermes cubicoceps*, of which there was not satisfactory material. The dimensions of the mastigotes in those hosts corresponded to those in the other hosts. The length and width averages and ranges are as follows:

[Size in μm of *Calonympha grandis* in twelve different hosts]

[Host termite]	Length	Width
Rugitermes panamae	69 (42–90)	42 (26–66)
Calcaritermes brevicollis	67 (37–107)	55 (33–88)
(exceptionally large specimens: 123 by 70 μm and 140 by 108 μm		
Calcaritermes emarginicollis	63 (33–107)	52 (25–68)
Calcaritermes nearcticus	75 (39–115)	58 (28–87)
Calcaritermes nigriceps	59 (37–110)	44 (27–68)

Calcaritermes sp. T-545	58 (31–101)	44 (30–77)
Calcaritermes sp. T-548	59 (44–75)	43 (33–58)
Glyptotermes guianensis	54 (37–81)	39 (25–50)
Glyptotermes perpavus	77 (41–117)	51 (34–78)
Proneotermes perezi	78 (55–112)	56 (34–82)
Rugitermes athertoni	50 (31–62)	36 (23–66)
Rugitermes kirbyi	79 (48–119)	37 (22–60)
Overall range	31–140	22–88

Several older and separately made sets of measurements were available. These are given as an example of the differences in results obtainable in measuring an arbitrarily selected small sample of the populations and consequently of the absence of significance in records to the fraction of a micron or calculation of statistical error. Fifty specimens of *Calonympha* from *Rugitermes panamae* gave an average of 76 by 45 μm, and ranges from 47 to 116 μm in length and 31 to 63 μm in width. In every figure there are differences of several microns from those obtained in the later set of measurements. Measurements of 50 specimens of *Calonympha* from *Calcaritermes brevicollis* gave an average of 68 by 50 μm, ranges from 45 to 92 μm in the length and 25 to 71 μm in width in these specimens. Other specimens extended the length minimum to 41 μm and the maximum to 123 μm. Here again there are differences of many microns in the figures obtained on the two independent occasions.

The number of akaryomastigonts varies greatly. In *Calonympha angusta* from the type host the estimated number is most often within the range of 60 to 150 and runs as high as 200 or more. In specimens from *Calcaritermes brevicollis* the range is from 20 to 150. In some groups of mastigotes only smaller numbers may be found, as for example in a slide of *Calcaritermes* on which numbers of 25 to 40 were found without greater numbers, whereas on another slide from the same termite colony the number ran up to 100 or more. The proportion between the number of akaryomastigonts and karyomastigonts is also variable. Often there are four times as many akaryomastigonts. *Calonympha angusta* from *Calcaritermes* (T-548), with not more than 10 karyomastigonts, had 50 to 100 or more akaryomastigonts. In *Calcaritermes nigriceps* the mastigotes often had about an equal number of the two types of mastigonts, or sometimes even more karyomastigonts than akaryomastigonts. But the higher numbers of the latter in many of the mastigotes in *Calcaritermes nigriceps* agreed with the situation prevailing generally in the species.

The number of karyomastigonts is usually small or moderate and shows much difference in range in different hosts. Figures obtained by counting the number of nuclei in 25 specimens from each host are:

[Number of karyomastigonts in *Calonympha angusta* from various hosts]

[*Host Termite*]	Average	Range
Rugitermes kirbyi	28	15–50
Calcaritermes brevicollis	29	9–50
Calcaritermes nearcticus	38	11–75
Calcaritermes nigriceps	34	20–60
Calcaritermes sp. T-545	9	3–25
Calcaritermes sp. T-548	6	4–10
Glyptotermes angustus	9	5–20
Glyptotermes perparvus	22	8–40
Proneotermes perezi	31	15–40
Rugitermes athertoni	19	8–50
Rugitermes kirbyi	44	20–60
Rugitermes magninotus	17	8–40

If one selected two specimens with about the same number of akaryomastigonts, one from *Calcaritermes* sp. (T-548) with a very small number of karyomastigonts and large nuclei and the other from *Rugitermes panamae* with five times the number and nuclei of much smaller size, they would seem logically to belong to different species. However, *Calcaritermes* sp. (T-545), also of El Salvador, has similar mastigotes with few large nuclei, intergrading with others with more and smaller nuclei, that extend well into the range of those in other hosts of *Calonympha angusta.* According to Dr. Alfred Emerson, the two termite hosts may be the same species or may differ only superficially. No definitive separation can be made in the whole complex on the basis of the actual and proportionate numbers of akaryomastigonts and karyomastigonts, or of the size of nuclei. If all the hosts actually contain only the one species, it must be recognized that forms that may be of minor occurrence or are absent in some hosts constitute the whole or a greater part of the population in others. A similar situation exists in *Calonympha grassii.* The population of *C. grassii* found by Calkins (1936) in certain *Cryptotermes brevis* of Puerto Rico, and which he named *Calonympha cryptotermitis*, apparently consisted only of mastigotes with the usually restricted range of from 12 to 16 karyomastigonts, a number less than the minimal found in calonymphids from some other colonies of *Cryptotermes brevis.*

There is a general plan of arrangement of karyomastigonts in a belt posterior to the zone of akaryomastigonts, but the distribution is nearly always more or less irregular. When there is almost a complete circle, the spacing of the nuclei is irregular. The plane of the belt is uneven. Nuclei are often arranged in double or triple rows in some places or are massed and extended in irregular

groupings. In some specimens nuclei or groups of nuclei are isolated in the posterior part of the body. Sometimes there is intermingling of karyomastigonts and akaryomastigonts, though in most specimens the two types are segregated. Occasionally one or several nuclei are situated in the anterior region among the akaryomastigonts. On the slide from *Glyptotermes perparvus* the extent to which intermingling took place was more marked than in any other material, though other slides in the same series did not show it to nearly the same extent. On this slide there were some mastigotes in which almost all of the karyomastigonts were mingled with akaryomastigonts in one mastigont zone.

The axostyles are grouped in a rather compact bundle that usually comes in contact with the membrane of the body at the posterior end. Here there may be an indentation of the posterior end. The density of the bundle varies. Often there are separated filaments along the periphery. In some specimens, observed especially in *Calcaritermes brevicollis* and *Calcaritermes nigriceps*, there is a tapered projecting cusp extended a few microns beyond the posterior end of the body.

In the mastigont region the axostyles turn abruptly away from the median axis at about the same level as that which they reach at the periphery. They separate at different levels along the central column, instead of spreading out from a single region. A dense bundle of filaments extends forward almost to the anterior end, diminishing in diameter as a result of giving off axostyles along the way. The most anterior axostyles are separated by only a few microns from the anterior end.

The upper parts of the axostyles are expanded, elongated capitula. The relationship of the capitulum to the rest of the axostyle is in position somewhat comparable to the blade of a hockey stick in relation to the handle. The capitulum is round in section. The axostyle commonly leaves the central column at an angle extending forward, about 45° or less from the column. After a distance, it turns rather abruptly to pass to the periphery, in a direction that is more or less transverse in most of the akaryomastigonts. In the apical akaryomastigonts it is a more forward direction, and sometimes, especially in the karyomastigonts, the direction may be somewhat backward. At this bend the expansion into the capitulum begins. From near its origin to the end the capitulum is cylindrical and maintains about the same diameter up to the broad, blunt end. In the posterior mastigonts the length of the capitula is almost 8 to 10 μm. In the more anterior akaryomastigonts the capitula becomes narrower and shorter, until in the apical ones they are scarcely developed at all.

The capitula are covered with bodies that, in iron hematoxylin, stain as small granules and in silver impregnation show as close-set oval corpuscles

whose margins alone are blackened and that are lined up in one or several rows. In the akaryomastigonts the corpuscles extend as far as the kinetosome at the end of the capitulum. In the karyomastigonts they can be seen only as far as the posterior end of the nucleus.

The kinetosomes of the posterior akaryomastigonts may be 25 μm or so from the place where the axostyle separates from the axial bundle, whereas the apical kinetosomes are at a distance away of only about 3 or 4 μm. The kinetosome is single and has the same size in all of the mastigonts. Next to the kinetosome is a narrow, elongated cresta, which is a minute inconspicuous structure less than 0.5 μm long. The separate undulipodium often extends along it, as is usual in these mastigotes. The size of the cresta is the same in all mastigonts, and this is also true of the four undulipodia in each mastigont.

The parabasal bodies differ greatly in shape and size in different mastigonts. Those of the apical akaryomastigonts are minute granules 0.5 μm or less in diameter. In akaryomastigonts situated more posteriorly the parabasals are increasingly larger and become elongated in form, reaching 2 μm or sometimes as much as 3 μm in the more posterior ones. These bodies are closely applied to the anterior part of the capitulum of the axostyle. The parabasal bodies of the karyomastigonts are usually markedly larger and distinctive in position. They are disc-shaped or elongated to 3 μm or more. Often they measure 4 or 5 μm in length, and in some specimens in some hosts as much as 6 μm. Often the rod-shaped parabasal bodies extend away from the nucleus, so that only at one end does it come in contact. It may be extended more or less in line with the nucleus but in the opposite direction, or it lies at the right or some other angle to that axis. It is not always extended away from the nucleus, however. Sometimes it is applied for the whole or part of its length to the nuclear membrane. Specimens with the entire parabasals applied to the nuclei are more frequent in some hosts than in others; they were frequent, for example, in *Calonympha angusta* of the *Calcaritermes* spp. from El Salvador. In *Calonympha* from these hosts also the karyomastigont parabasals often had a relatively small size, but they graded into other material more typical of the species.

The nuclei are spherical, and a clear space separates the nuclear membrane and the central chromatin mass. There are large size differences. In the mastigote occurring in *Calcaritermes* from El Salvador, when only five or fewer nuclei are present, the diameter may be as great as 7 μm or more. One specimen had two nuclei 10 μm in diameter, but that is highly exceptional. The nuclei have a diameter of 4 to 6 μm in most specimens of *Calonympha angusta*; occasionally some are found that measure somewhat less than 4 μm.

Cytoplasmic spherules are inconstant in occurrence. None has been seen in

specimens from the four species of *Rugitermes*. In *Calcaritermes* they were present in mastigotes in *Calcaritermes nigriceps*, in the termites from El Salvador, and in some material from *Calcaritermes brevicollis*, but they were not found in mastigotes from *Calcaritermes emarginicollis*. *Calonympha* of *Cryptotermes cubicoceps* usually had the spherules. They were present in some specimens from *Glyptotermes guianensis*, but not in others on the same slides. The same was true of calonymphids from *Calcaritermes brevicollis*. In some material all the spherules were usually small and grouped around the posterior part of the bundle of axostyles. In other material there was in addition usually one, sometimes more, relatively large spherule. In the mastigotes of *Procryptotermes [Proneotermes?] perezi* the spherules were best developed, being numerous and general in occurrence. In the material from Costa Rica they were relatively small in size, but in the material from El Salvador there were numbers of large ones. In the preparations from *Proneotermes perezi* these spherules were more or less impregnated in the preparations stained with protargol, but some preparations similarly treated from other hosts failed to show spherules that presumably were present.

Calonympha angusta from the species of *Calcaritermes* stained by protargol often showed rather large rods in the cytoplasm. Only the periphery of the rods is impregnated. Larger rods have a length of 11 μm or more, and these may occur in chains 25 μm or more long. Often there are two rods in tandem, two parts connected by a constriction. Some rods are constricted in more than one place; others show indications of cross-partitioning. Shorter rods may range down to 3 μm or less in length. The rods may occupy all parts of the cytoplasm to the level of the axostyles bending away from the bundle. They have been found in mastigotes of several different hosts, but they are not consistent in occurrence. Occasionally, as in some preparations from *Calcaritermes emarginicollis* and *C. nearcticus*, they are found in all specimens. In other preparations, as those from *Calcaritermes brevicollis* and *Calcaritermes* (T-544) from El Salvador, they can be demonstrated only in some specimens, whereas others contain small silver-impregnating rods and granules. In other specimens, in all of some preparations, no bodies are shown in the cytoplasm.

The rods described above have not been found in *Calonympha* from hosts belonging to other genera. Instead small granules and small rods may be present. These have been found in *Calonympha angusta* from *Rugitermes panamae, Rugitermes athertoni, Rugitermes kirbyi, Cryptotermes cubicoceps*, and *Proneotermes perezi*. Other termite species showed no calonymphids with silver-impregnated intracytoplasmic bodies.

Spirochetes, about 10 μm long, often adhere to the postmastigont part of

the body. In some specimens they form a dense coat over this whole region; in others they are restricted to the posterior end. There appear to be some specimens on which spirochetes are few or absent, but they adhere to *Calonympha angusta* in all of the hosts. At the posterior end of some specimens there are rods about 3 to 6 μm or more long, adherent by one end among the spirochetes. These have been found especially in *Rugitermes magninotus* and *Rugitermes panamae*. They are not present on any mastigotes from some hosts. [A generalized drawing of *Calonympha*, based on light micrographs, and reconstructed from electron micrographs is shown above in Fig. 2, page 13.]

***Stephanonympha spinosa* sp. nov. (Plate III: e, f, g)**

Type host – *Cryptotermes queenslandis* (Hill). S.W. Australia.

Diagnosis. Ellipsoidal; length ?? (56 to 100) μm; width ?? (33 to 90) μm; mastigonts number 60 to 300; usually > 180, arranged in 8 to 12 irregular transverse rows; occupying one-quarter to one-half of the cell length; cresta conspicuous, thorn-like, and about 2 μm long; parabasal body small rod applied to nuclear membrane at or near the anterior end; bundle of axostyles compact or loose and may end as truncate terminus, or there may be a posterior indentation; nuclei ellipsoidal and 3 to 4 μm long; large central chromatin mass with nucleolus; nuclei in contact with kinetosome; dense wood particles present; one large and several small spherules usually present; spirochetes 10 μm long adherent to posterior end; no adherent rods. In some species spirochetes are present only in the region around the end of the bundle of axostyles. In other specimens they are spread more widely over the posterior end; they are sparse, if present at all, on the sides of the body.

***Stephanonympha vescula* sp. nov. (no plates)**

Type host – *Neotermes tectonae* (Dammermann). Indonesia.

T-4527. Kateman, Sumatra.
T-4531. Kateman, Sumatra.
T-311. (Cleveland-Collier). Djember, Java.
T-4565. Telewa, Java.

Additional hosts –

Neotermes dalbergiae (Kalshoven). Java.

T-4568. Bandjar, West Java.
T-4569. Bandjar, West Java.
T-4532. Bandjar, West Java.
T-326. (Cleveland-Collier). Java.

Neotermes sonneratiae Kemner. Java.
T-4573. Angke, near Batavia.
T-4574. Angke, near Batavia.
Glyptotermes concavifrons Krishna & Emerson. Java.
T-4567. Bandjar.
T-4571. Bandjar.
Glyptotermes kirbyi Krishna & Emerson. Sumatra.
T-4538. Kateman.
Glyptotermes lighti Krishna & Emerson. Marshall Islands.
T-644. (J. Marshall). Arno Atoll.
T-639. (J. Marshall).

Diagnosis: Evenly ellipsoidal in shape; length 15 to 60 μm; width 15 to 48 μm; number of mastigonts 8 to 80, with overall average of 25; mastigonts occupy one-sixth to one-fourth of the length; mastigonts arranged in three or four spiral rows, but organization often loose; kinetosome large and in contact with both nuclear membrane and cell membrane; cresta slender and a little longer than diameter of kinetosome; parabasal body rounded or elongated with a length of 1 to 2 μm; axostyles gathered into slender bundle shortly below mastigont region; nuclei from 1 to 1.5 μm wide and 1.5 to 3 μm long, the larger being more typical; central chromatin mass dense and occupies most of space within nuclear membrane; cytoplasm filled with wood particles; usually no spherules present; spirochetes adhere to posterior ends of some specimens, usually in depression that commonly occurs at point where axostyle bundle reaches body wall; some specimens have stout rods adhering to posterior end.

The mastigote is relatively small in size and rather evenly ellipsoidal in shape. Specimens from *Neotermes tectonae* ranged in length from 16 to 52 μm, in width from 15 to 46 μm, averaging 33.5 by 27.8 μm. Those in *N. dalbergiae* ranged in length from 19 to 60 μm, in width 18 to 48 μm, averaging 38.1 by 29.7 μm. Those of *N. sonneratiae* ranged in length from 21 to 55 μm, in width 16 to 36 μm, averaging 31.6 by 27.4 μm. Almost all the specimens are somewhat elongated, but often the width is only a little less than the length. The posterior part of the body is not narrowed or protruded, as in some specimens of certain other species of the genus.

The number of mastigonts is comparatively small for *Stephanonympha*, counts averaging only 30 in *N. tectonae*, 38 in *N. dalbergiae*, and 32 in *N. sonneratiae*. The range in the species in the three hosts was from a rare minimum of 8 to a maximum of about 80. The occurrence of many specimens with fewer than 25 nuclei is a noteworthy feature of *Stephanonympha vescula*.

The single, large kinetosome is in contact with both the nuclear membrane and the cell membrane. The undulipodia originate from the kinetosomes in the usual manner. The cresta is small and inconspicuous. In favorably stained iron-hematoxylin material it appears as a slender projection from the kinetosome. Its length is hardly greater than the diameter of the kinetosome.

The parabasal body is a rounded or somewhat elongated structure usually in contact for its entire length with the anterolateral surface of the nuclear membrane, though sometimes it extends away from it. Its greatest diameter is usually 1 to 1.5 μm, sometimes as much as 2 μm.

The axostyles usually gather into a bundle at about the level of, or a short distance posterior to, the posterior mastigonts. The relatively slender, compact bundle extends from that region to the posterior end of the body, where sometimes there is an indentation. Occasionally the axostyles are loosely instead of compactly aggregated, but that is exceptional. Often, especially upon superficial examination, the bundle appears to be homogeneous (and uniformly stainable), resembling the axostylar trunks of *Devescovina* and *Caduceia* that are found on the same slides. Closer inspection, of course, shows that the bundle is composed of many filaments.

On several slides from *Neotermes dalbergiae* (T-4532) most specimens of *Stephanonympha vescula* had a broader bundle of more loosely aggregated axostylar filaments, in the arrangement of which the usual laeotropic twist was apparent. Slender compact bundles occurred also, but exceptionally in this series of slides.

The nuclei vary in size, from about 1 by 1.5 μm in small mastigotes with few nuclei to 1.5 by 3 μm in large specimens. In the majority (or large proportion) of specimens the nuclei tend toward the larger size. The central chromatin mass is dense and occupies most or all of the space within the nuclear membrane. The kinetosome is in close proximity to the anterior end of the central chromatin mass.

The cytoplasm is densely filled with particles of wood. Unlike most other species of *Stephanonympha* the cytoplasm usually does not contain the spherules around the posterior part of the axostyles or elsewhere. No spherules were seen in any material from the three species of *Neotermes*; but some small spherules were present in the specimens from *Glyptotermes*.

Spirochetes, which in fixed material are often about 10 μm long, adhere in a dense tuft at the posterior end of most but not all specimens. Usually they are restricted to a limited region where the axostyle bundle ends, in which place there is often a depression. Sometimes the spirochetes are extended more broadly over the posterior part of the body but not on the sides. In

one series of preparations from *Neotermes dalbergiae* the mastigotes also bore stout, slightly curved rods, 4 or 5 μm long, adhering by one end.

The *Stephanonympha* in the species of *Glyptotermes* listed above from Java and Sumatra is somewhat doubtfully identified as *S. vescula.* It has, like the specimens in *Neotermes*, a rather small number of mastigonts and a bundle of axostyles that is usually compact and slender. In *Glyptotermes, Stephanonympha* is, as in *Neotermes*, associated with *Calonympha*, an unusual combination in the termites we have studied [Fig. 5].

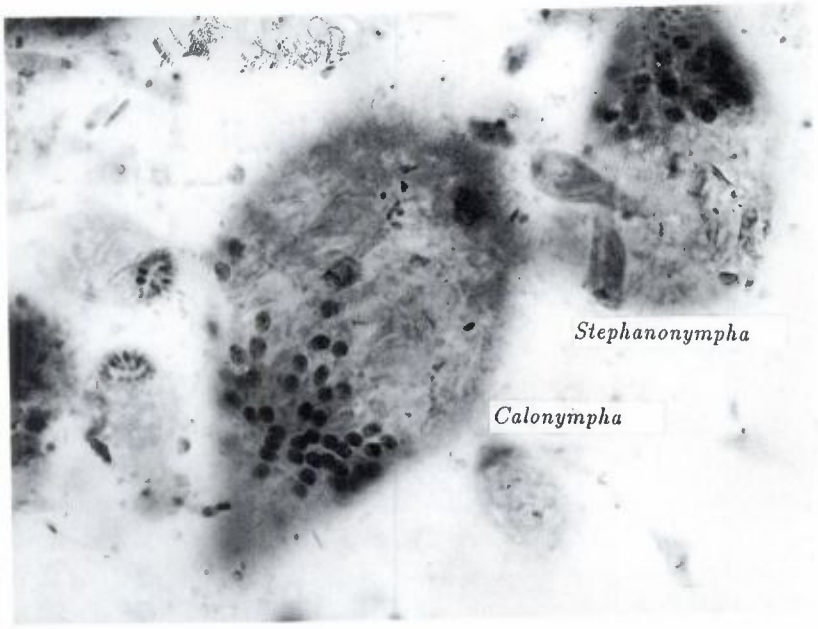


Figure 5. *Calonympha* and *Stephanonympha* from *Glyptotermes*, Sumatra. Harold Kirby's protargol preparation made c. 1950.

Stephanonympha in *Glyptotermes lighti* from the Marshall Islands also seems to be *S. vescula* in all taxonomically significant characteristics. It is comparable with this species in its moderate size and in the relatively small number, as well as in the arrangement, of mastigonts. The bundle of axostyles is relatively slender, since the number of its component filaments is small. In many specimens the bundle is as compact as that in *Stephanonympha vescula* or *Neotermes tectonae*; in others the axostyles are more widely dispersed. In

many specimens on the slides the axostylar bundle projects for a short distance from the posterior end. The parabasal body is slightly elongated and sometimes longer than the nucleus.

In some protargol-stained preparations of *S. vescula* from *Glyptotermes lighti* the cresta was well impregnated. It has an elongate triangular, somewhat curved shape and a length of about 1 to 1.5 μm. It is visible also in suitably stained iron hematoxylin-stained slides, but in this latter material it is not so conspicuous and does not appear so large. Some silver-stained preparations showed clearly the group of three anterior undulipodia and the single undulipodium extending along the exterior edge of the cresta for either a part or all of its length.

The protargol preparations show in the cytoplasm numerous deeply impregnating bodies. These are sometimes granules or rows of granules but usually are rods of variable length, often curved. Many are attenuated toward one or both ends. These do not appear in all specimens on some slides and in some specimens on other slides; or they may be consistently present in all *Stephanonympha* individuals in some preparations. Their apparent absence in some preparations may be due to failure to impregnate.

There are no adherent microorganisms on *Stephanonympha vescula* in *Glyptotermes lighti.*

Calonympha umbella sp. nov. (no plates)

Calonympha patella Brown *nomen nudum,* 1941, *J. Tenn. Acad. Sci.* 16(4):358.

Type host – *Neotermes tectonae* (Dammermann). Sumatra.
T-4531. Java.
T-4527. Sumatra.
T-4542. Sumatra.

Additional hosts –
Neotermes dalbergiae (Kalshoven). Java.
T-4521. Java
Glyptotermes kirbyi Krishna & Emerson. Sumata.
T-4538. Sumatra.

Diagnosis: Ovoid and broadly rounded anteriorly; length 62 (43 to 83) μm; width 46 (28 to 56) μm; average number of mastigonts 12; one-fifth to one-third of body length covered by mastigonts; nuclei arranged in irregular circle; akaryomastigonts spread equally a short distance posterior to karyomastigonts and arranged in a broad dome; kinetosome a distinct single body; cresta little

longer than kinetosome; parabasal bodies disc-shaped, equal in size throughout the individual, 1 to 2.5 μm in length with a thickness of about 0.5 to 1 μm; nuclei spherical, 4 to 6 μm in diameter; interior of nucleus occupied by granules; spherical bodies present in cytoplasm; spirochetes adhere in dense cluster to the posterior end; some specimens contain short curved rods adherent to posterior part of body.

The general shape of *Calonympha umbella* is variable but tends to be broadly rounded anteriorly. Brown (1941a) found the length to be 37 to 126 μm, with an average of 71 μm, and the width to be 25 to 117 μm, with an average of 56 μm. Our measurements from *Neotermes tectonae* of Sumatra averaged 62 μm in length, with a range of 43 to 83 μm, and 46 μm in width, with a range of 28 to 56 μm. In *Neotermes dalbergiae* of Java the length averaged 84 μm, ranging from 51 to 140 μm. The width from this host averaged 63 μm and ranged from 39 to 78 μm. In *Glyptotermes* sp. (T-4538) from Java the average length was 69 μm, ranging from 46 to 88 μm; the average width was 50 μm, ranging from 33 to 80 μm. The number of nuclei was 12 as an average in *Calonympha umbella* of *Neotermes tectonae* of Sumatra. In *Neotermes dalbergiae* the average number of nuclei was 16, and in *Glyptotermes* sp. it was 8. In the several hosts the portion of the body occupied by the mastigonts varied from one-fifth to one-third.

Nuclei are not arranged in regular rows. Those with a small number of nuclei tend to be more regular. In some specimens more than half of the nuclei may be crowded to one side. The akaryomastigonts are distributed at the apex of *Calonympha umbella* as well as elsewhere, in contrast to *Calonympha angusta*, where the akaryomastigonts are not found in the extreme tapered apical part, or are much reduced in size there and crowded together.

The mastigonts of *Calonympha umbella* spread out in a very different manner from those of *Calonympha grassii* and *Calonympha angusta.* Instead of a central column extending to the anterior end and giving off axostyles almost to that end, all the axostyles or groups of axostyles begin to spread equally a short distance posterior to the level of the karyomastigonts. That manner of spreading is more like that in *Stephanonympha* species. The peripheral parts of the mastigonts are arranged in a broad dome, or sometimes the sides are somewhat narrowed. The capitula of all axostyles are equally spaced and of about the same size.

The parabasal body has the form of a biscuit with the flat side applied to the capitulum of the axostyle close to the anterior end. The akaryomastigont parabasals are usually consistent in size and shape. In many specimens

the parabasals of the karyomastigonts greatly resemble those of the akaryomastigonts. The parabasals range from about 1 to 2.5 μm in length, with a thickness of about 0.5 to 1 μm.

The nuclei are spherical and about 4 to 6 μm in diameter. The interior of the nucleus is occupied by granules that destain to become paler than is usually true of such granules. At the periphery is a deep-staining, usually elongated body set off by a clear zone.

The kinetosome is a single moderate-sized body that stains deeply. It is usually less than 1 μm in diameter.

The separation of the cresta from the kinetosome can be seen only in favorably stained and oriented specimens. It often appears as a narrow projection from the kinetosome. It is but slightly longer than the kinetosome. This vestigial cresta, slender throughout its length, is slightly thicker toward the base. The separate undulipodium extends from the kinetosome along the cresta.

Light brown spherical bodies are present in the cytoplasm. There may be one or several ranging in diameter from 7 to 12 μm.

Spirochetes occur in a dense cluster adherent at the posterior end, or sometimes over a broader zone but not extending over the whole postmastigont region.

On some protargol-stained slides there are numerous short, curved rods adherent to the surface of the posterior part of the body among the spirochetes.

4. Discussion and Summary

[Missing from Kirby's original manuscript]

To summarize this information Table 1 presents Yamin's compilation of termite hosts and their calonymphid symbionts. The additions permitted by publication of this posthumous paper are in Table 2. The characteristics of calonymphids recently used for cladistic analysis by Brugerolle and his colleagues (Viscogliosi et al., 1993) are presented in Table 3, and a summary of the classification lists the genera in Table 4. The species names, listed alphabetically by termite host, are in Tables 1 and 2. For the comprehensive alphabetical list of species of the two genera Calonympha and Stephanonympha see Table 5.

My view, in retrospect, of the importance of this work for the origin of eukaryotic cells (Margulis, 1993) is summarized as follows.

Protistology and taxonomy. *Since Kirby worked on these obscure organisms, molecular phylogenetics has been established as a field of biology, and protist taxonomy has taken on a new importance. We now realize that the earliest*

eukaryotes, including the ancestors to animals, were like Kirby's calonymphids: mastigotes lacking mitochondria that thrived in anoxic environments. Although the family Calonymphidae was erected in 1911 to contain these protists on the basis of their morphological uniqueness and hindgut habitat, it was really Kirby's work that established the position of the six genera and their relationship with devescovinids and trichomonads in the biological world. The group apparently speciates and complexifies by Kirby's process of "mastigont multiplication." In anoxic conditions reminiscent of the atmosphere during the Proterozoic eon, these protists, in the later Phanerozoic eon, co-evolved with their dry-wood-eating Isopteran hosts. Since both the animal host and the calonymphid genera are entirely restricted to their mutual association, these mastigotes provide another example of the taxon-limited symbionts.

Evolution. *Here Kirby describes his "principle of mastigont multiplicity" showing how this series of protists, from devescovinids and trichomonads through the large-celled calonymphids, are related by varying numbers of reproductive sets of organelles, with and without nuclei.*

The kinetosome-centriole DNA. *Kirby's natural history studies document the uniqueness of calonymphids for cytological investigation. These relatively large protists, symbiotic in accessible quantities in well-known insects, normally produce "controls" and "experimentals" within the same cell that could optimally be used for seeking kinetosome-centriole DNA. The anterior portions of* Calonympha *or* Stephanonympha *cells tend to have a greater number of akaryomastigonts relative to karyomastigonts; thus some of their nuclear DNA reproduction is uncoupled and physically separate from that of the (akaryomastigont) kinetosomes. Nuclear DNA is in close proximity as part of the karyomastigont of the same cell. Kirby's observations permit the direct study of the reproduction of kDNA in the presence and absence of the nuclei in a natural system. Whereas* Chlamydomonas *cells have only a pair of kinetosome-centrioles per cell, we know from Kirby's and subsequently Brugerolle's work that calonymphids may have hundreds of kinetosome sets, each organized with (karyo-) and without (akaryo-) its nucleus. Thus, calonymphids make ideal material for probing with any putative kinetosome-centriole DNA (e.g., such as that reported by Hall et al., 1989).*

The possibility for definitive detection of kinetosome DNA using calonymphids is enhanced by the unpublished accomplishments of Michael Yamin. He isolated and developed the medium for growing one species of calonymphid (Coronympha octonaria from Incisitermes snyderi) in monoprotist culture.

Strict anoxic conditions are employed and cellulose serves as carbon source. Since 1981 C. octonaria has been subcultured and maintained by John Breznak (Michigan State University) and by David Odelson (Central Michigan University). Still available, this calonymphid would be an appropriate protist for continued pursuit of centriole-kinetosome DNA. Kirby would have been pleased to learn that analysis of the sequence of nucleotides in the gene coding for the small subunit ribosomal RNA unequivocally places C. octonaria with other trichomonads (John Gunderson, Gregory Hinkle and others, manuscript in preparation). No other calonymphid sequence data is available. I hope this publication of Kirby's work will stimulate renewed interest in this remarkable family of zoomastiginid protists.

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* Many not cited in text, others cited in text not listed. These are what Kirby provided in his unfinished manuscript.

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6. Note

Harold Kirby is remembered for his meticulous work by John O. Corliss, Ernst Mayr, Ledyard Stebbins, Alistair M. Stewart, William Trager, and E.O. Wilson, none of whom are familiar with details of calonymphid biology.

We have made the following changes in preparing this manuscript: We hyphenated words according to current standards, corrected basic punctuation mistakes, corrected "that" to "which" and vice versa as appropriate, converted fractions to decimals, and substituted "μm" for "μ," "%" for "percent," "by" for "x" in the statement of dimensions, and "to" for "–" in measurement ranges. Terminology was updated as follows:

Kirby	here
basal bodies	*kinetosomes*
daughter cells	*offspring cells*
flagella	*undulipodia*
flagellates	*mastigotes*
protozoa	*protists*
protozoa faunules	*protists, faunules*

The following terms used in this manuscript are conformed to modern definitions in the Illustrated Glossary of Protoctista *(Margulis et al., 1993):*

akaryomastigont: *Karyomastigont system (q.v.) lacking its nucleus, generally of calonymphids. Tend to lie anterior to the nuclei-bearing karyomastigonts.*

Calonymphidae: *Calonymphids. Family in phylum Zoomastigina. (See Tables 1–5, pages 26–31 above.*

centriole-kinetosomes: *See "kinetosomes" below.*

chromatin: *Eukaryotic DNA complexed with histone (and/or other basic proteins) to form the nucleosome-studded DNA strands that usually "condense" (coil and become deeply stainable) to form chromosomes during mitotic cell division.*

costa: *Nonmicrotubular striated intracellular rod, a periodic fiber, in zoomastiginids widespread in trichomonads and other parabasalids, motile in some. Also an attachment band, connected at both ends to coiled filaments that confer elasticity to the cortex in acantharian actinopods. Rib or ridge (e.g., foraminifera).*

cresta: *Fibrillar, noncontractile structure, limited to devescovinid and hypermastigote parabasalids. A strait or curved rod, it is thicker at the end where it meets the kinetosome of the trailing (recurrent) undulipodium. About 0.5 μm, this organelle which stains with iron hematoxylin, is sometimes attached to the recurrent undulipodium.*

devescovinid: *Protoctist member of the family Devescoviidae, order Trichomonadida, class Parabasalia, phylum Zoomastigina. Uninucleate mastigote with four undulipodia per karyomastigont, a cresta, stationary axostyle, no costa or pelta. Sexuality is not known in the group; all are symbiotic in insects.*

karyomastigont: *Intracellular organellar complex found in diplomonads, trichomonads, and other mastigotes that includes the nucleus and attached organelles: minimally kinetids with their kinetosomes and undulipodia. May also include parabasal bodies (Golgi), rhizoplast, fibrillar cresta, axostyles, costa, undulating membrane, and pelta. A karyomastigont generally includes a nucleus attached to four undulipodia. Maximally, each karyomastigont may include all of the attached structures, in species-specific ratios.*

kinetid: *Basal apparatus; undulipodial ("flagellar") apparatus. Kinetosomes and their associated tubules and fibers present in all undulipodiated cells; unit of organization of the ciliate cortex. The functional organellar complex, including undulipodia, is usually responsible for locomotion. Synonyms include: basal apparatus, flagellar apparatus; flagellar root systems; proboscis root; root fiber system; undulipodial apparatus; kinetosomal territory; ciliary corpuscle. Kinetids always consist of at least one kinetosome, but may have pairs or occasionally more than two kinetosomes (e.g., they may be dikinetids or polykinetids). Structures associated with the kinetosomes of ciliates usually include cilia, unit membranes, alveoli, kinetodesmata, and various ribbons, bands, or bundles of microtubules (e.g., postciliary microtubules and some nematodesmata). Root microtubules of kinetids may be laterally associated microtubules that originate at kinetosomes in definite numbers and follow a defined path within the cell (e.g., ciliates). Some kinetids are also composed of microfibrils, myonemes, parasomal sacs, mucocysts, or trichocysts. Details of the kinetid are essential for taxonomic and evolutionary studies of motile protoctists. The mastigont system of trichomonads (i.e., akaryomastigonts) is an example of a complex kinetid. May also include parabasal bodies (Golgi), rhizoplast, fibrillar cresta, axostyle, costa, undulating membrane, and pelta.*

kinetosomes: *Basal bodies of eukaryotes. Intracellular organelles not membrane-bounded, characteristic of mastigotes, sperm, and all other undulipodiated cells. Microtubule structures, cylinders about 0.25 μm in diameter and up to 4 μm long. All undulipodia are underlain by kinetosomes with microtubules organized in the [9(3)+0] array. Kinetosomes differ from centrioles (which share characteristic cross sections of a circle of nine triplets of microtubules) in that from them extend the [9(2)+2] shafts. The basal kinetosome is necessary for the formation of the undulipodium shaft, called the axoneme. The rotary motor disk of bacterial flagella, an entirely different far smaller structure, is often called "basal body"; therefore the term kinetosome, because of its precision, is preferable. Mitotic centrioles become kinetosomes in the development of cells. In some protists when the axoneme is resorbed or sloughed off, the kinetosome may become a centriole; for this reason the double term "centriole-kinetosome" is sometimes employed.*

parabasal body: *Golgi apparatus anterior in the cell which defines the class Parabasalia. Located near the kinetosomes and their associated structures, this body (derived from endoplasmic reticulum and formed anew after each cell division) generally resides near the nucleus. More compact than in other cells, it probably has a secretory function.*

parabasal filaments: *Striated fibers or lamellae with thin lines (e.g., some have a periodicity of about 42 nm) that emerge from the kinetosomal complex and extend to the parabasal bodies. May function in the segregation of parabasal (Golgi) materials.*

paradesmose: *Cell structure comparable to a thin, extranuclear mitotic spindle in many mastigotes. It links two sets of polar kinetosomes during mitosis (e.g., paradesmose in some prasinophytes is composed of a thin bundle of microtubules).*

pelta: *Crescent-shaped microtubular structure associated with the anterior portion of the axostyle in parabasalids and pyrsonymphids. Capitulum of axostyle.*

undulipodium: *Eukaryotic motility organelle: kinetosome plus associated axoneme. Cilium. Sperm tail; cell-membrane-covered motility organelle usually showing swimming, feeding (or sensory) functions and composed of at least 200 proteins including tubulin and dynein. Limited to eukaryotic cells, [9(2)+2] microtubular axoneme is usually covered by plasma membrane. Includes cilia and eukaryotic "flagella." Each undulipodium invariably develops from its kinetosome. Contrasts in every way with the prokaryotic organelle shaft or flagellum, which is a nonmotile structure composed of a single protein. (This belongs to the class of proteins called "flagellins" and is driven by an ion-pumping rotary motor). Undulipodia in the cell biological literature are still referred to by the confusing term "flagella."*

7. Biographical note[1]

The death of Harold Kirby on February 24, 1952 marked the untimely end of a brilliant career devoted largely to the study of parasitic Protozoa. Born in Tusket, Nova Scotia, Kirby received his undergraduate training at Emory University. His scholastic ability and promise were soon recognized by Professor R.C. Rhodes, who aroused Kirby's interest in the Protozoa and sent him on to Berkeley for graduate training in the laboratory of Professor C.A. Kofoid. After receiving the Ph.D. degree in 1925, Kirby served as Instructor in Zoology at Yale University. He came back to join the Department of Zoology on the University of California's Berkeley campus in 1928, and he remained there for the rest of his life. In 1948, he became Chairman of the Department, a position he held at the time of his death.

Kirby's biological interests extended beyond the laboratory, for he was a naturalist with a keen enjoyment of field work which he pursued in trips to the Fanning Islands, to Panama, as a Guggenheim Fellow to Africa, Madagascar and Java, and in his work at the Hastings Reservation of the University of California.

In all of his scientific investigations, Harold Kirby was an exceptionally careful and accurate worker. His conclusions were always on ample evidence and his contributions to the science of protozoology were of fundamental importance. His care in observation and his enthusiasm for research were transmitted to his graduate students, who are carrying on in his tradition.

He served as Vice-President of the [American Society of Parasitologists] in 1946 and...organized the very successful symposium presented at the annual meeting that year.

Among the other honors which came to him were his election as a Fellow of the California Academy of Sciences in 1947, and as Vice-President of the Society of Protozoologists in 1952. He represented the American Society of Zoologists at the 13th International Congress of Zoology in Paris in 1948, serving also as an alternate member of the International Commission on Zoological Nomenclature. He served on the editorial board of the *Journal of Morphology* and as Chairman of Editors of the University of California Publications in Zoology.

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[1] Excerpted from Ball and Hall (1953). See Fig. 4, page 15 above.

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