

Redescription of *Cryptobia helicis* Leidy, 1846 (Kinetoplasta: Bodonea: Cryptobiidae), Disposition of Flagellates Mistakenly Assigned to This Species, and Description of a New Species from a North American Pulmonate Snail

Eugene N. KOZLOFF

Friday Harbor Laboratories, University of Washington, Friday Harbor, Washington, USA

Summary. *Cryptobia helicis* Leidy, 1846, the type species of the genus, is redescribed on the basis of material from the seminal receptacle of *Neohelix albolabris albolabris*, the first of three host pulmonate snails mentioned by Leidy. The flagellate from *Triodopsis tridentata*, the second host mentioned by Leidy, is very different, and is described as *C. innominata* sp. n. The *Cryptobia* in *Anguispira alternata*, the third host designated by Leidy, is also distinct, but cannot properly be described until more material is available. The extensively studied cryptobias in *Helix pomatia* and *H. aspersa aspersa*, long considered to be *C. helicis*, are very different from true *C. helicis* and *C. innominata*. The name *C. helicogena*, originally proposed as *Trypanoplasma helicogena* Kühn, 1911, is applicable to the flagellate in *H. pomatia*, and tentatively also to the one in *H. aspersa aspersa*.

Key words: Bodonea, *Cryptobia helicis*, *C. innominata* sp. n., *C. helicogena*, Kinetoplasta, taxonomy.

INTRODUCTION

The description of *Cryptobia helicis* by Leidy (1846) was based on material taken from the seminal receptacle of three species of terrestrial pulmonate snails collected in the vicinity of Philadelphia, Pennsylvania, USA. Two of the snails, *Neohelix albolabris albolabris* and *Triodopsis tridentata* (referred to by Leidy as *Helix albolabris* and *H. tridentata*), belong to the family Polygyridae; the third, *Anguispira alternata* (also referred to as a species of *Helix*), is a member of the family Endodontidae. Soon afterward Leidy (1847a) requested the permission of the Academy of Natural

Sciences of Philadelphia to replace *Cryptobia* with *Cryptoicus*, because a similar name, *Cryptobium*, had previously been used for a genus of beetles.

Leidy published three other papers dealing with *C. helicis*, one of them (1847b) being nearly the same as his first. In the text of this paper, however, he used *Cryptoicus* in place of *Cryptobia*, and he enlarged the list of hosts, mentioning *Bulimus decollatus* (now called *Rumina decollata*) and "several other species of *Helix*, viz. *Helix elevata* and *H. thyroidus*" (now called *Mesodon elevatus* and *M. thyroidus*). The genus *Rumina* is in the family Achatinidae, and *Mesodon*, like *Neohelix* and *Triodopsis*, is in the family Polygyridae. The next paper (Leidy 1851) gave the name of the flagellate as *Bodo helicis*, a change proposed by Diesing (1850), and listed both *Cryptobia helicis* and *Cryptoicus helicis* as synonyms. The last mention of *Cryptobia* by

Address for correspondence: Eugene N. Kozloff, Friday Harbor Laboratories, 620 University Road North, Friday Harbor, Washington 98250, USA; Fax: (206) 543-1273; E-mail: ekoz@u.washington.edu

Leidy (1856) again merely listed *Bodo helicis* with its synonyms.

Diesing (1850) was the first author to have encountered a *Cryptobia* in a European snail. He found the flagellate to which he referred as *Bodo helicis* in *Helix nemoralis* (family Helicidae) at Vienna. Soon afterward, Keferstein and Ehlers (1860) reported on "Infusorien" occurring in the seminal receptacle of *Helix pomatia*. Their illustrations, like those of Leidy, show clearly that the organisms were flagellates. After the start of the twentieth century, various European authors became interested in cryptobias. Friedrich (1909), Jollos (1911), Bělař (1916, 1926), and Schindera (1922) published accounts of the structure, division, and other aspects of the biology of what all of them called *Trypanoplasma helicis*, and which all of them obtained from the seminal receptacle of *H. pomatia*. Kühn (1911) also used *Trypanoplasma* in place of *Cryptobia*. He examined a wide variety of terrestrial and freshwater snails, thereby enlarging the list of known hosts. In addition, he named and briefly described five species: *T. helicogena* (from *Helix pomatia*, at that time assigned to the subgenus *Helicogena*); *T. tacheorum* (from *Tachea nemoralis*, *T. hortensis*, and *T. austriaca*); *T. desertorum* (from *H. desertorum* and *H. desertorum* var. *hasselquitzki*); *T. rupestre* (from *H. cingulata*); and *T. limnorum* (from *Lymnaea stagnalis* and *L. palustris*). He thought the species in *H. aspersa* was perhaps *T. helicogena*, and that the flagellate in *H. arbustorum* was possibly *T. tacheorum*. Flagellates found in *H. lactea*, *H. muralis*, and *H. virgata* var. *variabilis minor* were not assigned to species. His opinion with respect to the status of the flagellate from *H. aspersa* will be dealt with in a later portion of this paper.

Kühn's (1911) descriptions and illustrations of species are too sketchy to firmly establish the individuality of any of them. Matthey (1923), who studied flagellates from several species of European snails and who also used the genus name *Trypanoplasma*, took a more conservative approach, pointing out that the criteria Kühn used for distinguishing species, primarily dimensions of the body and relative lengths of the flagella, were superficial. Matthey had noted that these characters varied in flagellates from one species of snail to another, but thought the variations might be due to physiological conditions in the seminal receptacles of different hosts. He suggested that Kühn's species might better be treated as subspecies of "*T. helicis*," then went on to say that even this seemed to be unnecessarily precise.

The type species of *Trypanoplasma* is *T. borreli* Laveran and Mesnil (1901), first found in the blood of *Scardinius erythrocephalus* and later in various other freshwater fishes. At least for flagellates from the seminal receptacle of pulmonate snails, however, *Cryptobia* has priority (Crawley 1909), and it has sometimes been applied to flagellates from the blood of fishes and amphibians as well as to those in snails. Brugerolle *et al.* (1979) pointed out that in spite of the remarkable similarity of flagellates from fishes and those from snails (the similarity is evident in electron micrographs as well as in intact specimens studied with the light microscope) the blood parasites are typically transmitted by leeches, within which there are stages rather different from those in the blood. They proposed, therefore, that the two genera not be synonymized. They even went a step further, suggesting that separate subfamilies (Cryptobiinae, Trypanoplasmatinae) be established. Woo and Wehnert (1983), however, found that "*Cryptobia*" *salmositica* can be transmitted from one rainbow trout to another without involvement of an intermediate host. Nevertheless, recent studies of kinetoplast DNA and RNA of various bodonid flagellates, including *Trypanoplasma borreli* and the *Cryptobia* from *Helix pomatia* (Lukeš *et al.* 1998, 2002; Doležel *et al.* 2000; Simpson *et al.* 2002) strongly indicate that *Trypanoplasma* and *Cryptobia* belong at least in separate genera.

My cytological study (Kozloff 1948) of what I assumed to be Leidy's *Cryptobia helicis* was based on material from the European *Helix aspersa aspersa*, now well established in some areas of North America. A *Cryptobia* found in *Monadenia fidelis*, a helicid snail native to the Pacific northwest region of North America, appeared to be similar, but this host was not so readily available as *H. aspersa aspersa* and my protargol impregnations of its flagellate parasites were not particularly good. At that time I had not seen material from any of the three snails in which Leidy found the parasites on which he based his description of *C. helicis*. Later, however, I was able to study flagellates from *Triodopsis tridentata* and *Anguispira alternata* collected near Ithaca, New York, and also from a snail identified as *Neohelix albolabris*, collected at Chapel Hill, North Carolina. The latter has recently been named *N. solemi* (Emberton 1988), on the basis of penis morphology and certain shell characters. The parasites in each of these three host species were different, and none of them was the same as the flagellate taken from *H. aspersa aspersa*. Thus it became clear that the most intensively

studied cryptobias, those from *Helix pomatia* and *H. aspersa aspersa*, must have another name. Unfortunately, my attempts to make satisfactory protargol impregnations of the cryptobias from *T. tridentata* and *A. alternata* failed. It was not until recently that parasitized *N. albolabris albolabris* and additional *T. tridentata* became available to me. Attempts to obtain more parasitized specimens of *Anguispira alternata* have not been successful.

Pyne (1959, 1960, 1967) was the first to describe the ultrastructure of the *Cryptobia* from *Helix pomatia*. A few years later, Brugerolle *et al.* (1979), for their comprehensive ultrastructural study of flagellates belonging to the *Bodo-Cryptobia-Trypanoplasma* group, used material from both *H. pomatia* and *H. aspersa* (almost certainly subspecies *aspersa*). They reported no differences between flagellates from these two host snails. Another study of ultrastructure of a *Cryptobia* is that of Current (1980), concerned to a large extent with the interesting way in which the flagellate is attached to cells in the wall of the seminal receptacle. Current's material came from a polygyrid snail collected in Nebraska and identified as *Triadopsis multilineata* (correctly *Triadopsis multilineata*), recently assigned to *Webbhelix* (Emberton 1988). The flagellate in this snail is probably much closer to the ones from the first two polygyrids examined by Leidy than to those occurring in *H. pomatia*, *H. aspersa aspersa*, and other European snails of the family Helicidae.

Another complication related to the problem under discussion here is that the genus name *Cryptobia* has been used for certain flagellates parasitizing the gills or digestive tract of fishes, and for a few other organisms found in various vertebrate and invertebrate hosts. Not all of them are necessarily close to flagellates of the *Cryptobia-Trypanoplasma* complex. Brugerolle *et al.* (1979) studied the ultrastructure of three species from fishes, one living on the gills of a freshwater fish, two inhabiting the digestive tract of marine fishes, and found the morphology of these flagellates to be similar to that of species of *Cryptobia* and *Trypanoplasma*. In reconstructions of the ultrastructure of *Cryptobia* from species of *Helix*, Brugerolle *et al.* (1979) showed the cytostome to be located much farther posteriorly than it is in the two fish cryptobias whose structure they also illustrate. Current (1980), however, showed the cytostome of the species of *Cryptobia* he found in *Webbhelix multilineata* also to be relatively far forward.

The purposes of this paper are to establish the identity of *Cryptobia helcis* from *Neohelix albolabris*

albolabris, to distinguish it from the flagellate in *Helix pomatia* and *H. aspersa aspersa*, to suggest a name by which the flagellate in these European snails can be called, and to describe the species parasitizing *Triadopsis tridentata*. It is not likely that any flagellates of fishes or of invertebrates other than pulmonate snails will have a bearing on the systematics of *C. helcis* and related flagellates that have been mistakenly assigned to it, so no further consideration will be given to them.

This study is based entirely on light-microscope observations. These, unfortunately, do not reveal details of some important structures that can be seen in electron micrographs. Nevertheless, techniques of staining and impregnation bring out enough details to make slide preparations useful for distinguishing species that are decidedly different, which is the case with the flagellates that will be dealt with here

MATERIALS AND METHODS

To prepare cryptobias for light microscopy, the most useful single method is impregnation with activated silver albumose (protargol). When it works successfully, it brings out the flagella and nucleus, as well as the shape of the cell, and sometimes what I described as the "aciculum" (Kozloff 1948). For fixation prior to impregnation, Hollande's fluid and 5% glutaraldehyde (not buffered) were used; the former gave best results. Staining with iron hematoxylin after both fixatives stained the kinetoplast, which is not impregnated by protargol, and the endosome and peripheral chromatin of the nucleus. Phase-contrast optics were helpful in making measurements of body form and flagella of living flagellates.

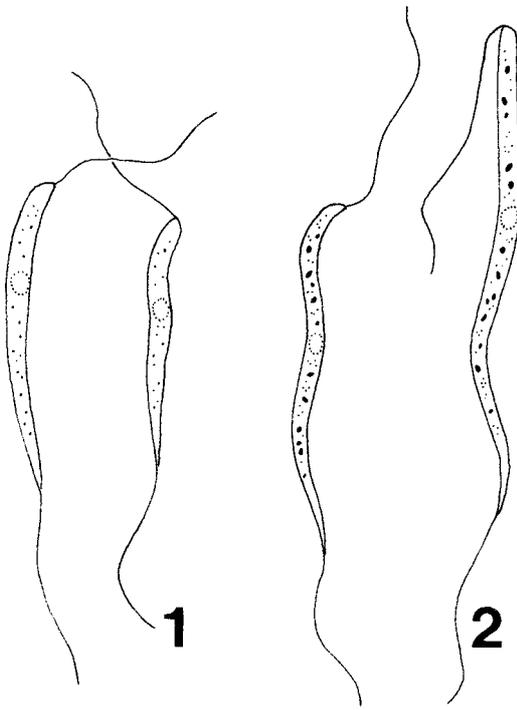
The specimens of *Helix aspersa aspersa* for my 1948 paper were collected in Berkeley and Oakland, Alameda County, California, USA; the slide preparations are still good. Additional specimens of *H. aspersa aspersa* were collected recently in El Cerrito, Alameda County. Fixed smears of the contents of the seminal receptacle of *H. pomatia* were sent to me from near České Budějovice, Czech Republic. A parasitized *Neohelix albolabris albolabris* was found near Newport, Perry County, Pennsylvania, USA and specimens similar to it, but now assigned to *N. solemi*, were collected in Chapel Hill, Orange County, North Carolina, USA. *Triadopsis tridentata* was found in Westmoreland County, Pennsylvania, USA.

RESULTS

Redescription of *Cryptobia helcis* Leidy, 1846 (Figs 1, 3-6)

Host: *Neohelix albolabris albolabris* (Say, 1816) (Polygyridae).

Because Leidy listed "*Helix*" *albolabris* first among the three hosts in which he reported the presence of



Figs 1-2. 1 - *Cryptobia helcis* Leidy from *Neohelix albolabris albolabris*; drawing of living specimens. 2 - *Cryptobia innominata* sp. n. from *Triodopsis tridentata*; drawings of living specimens.

C. helcis, the flagellate parasitizing this snail has been chosen as the type species of the genus. There is another good reason for doing this: Leidy's drawing of a mass of flagellates showed the body of most individuals to be comparatively plump and closer to the shape of specimens from *N. albolabris albolabris* than to the shape of those from *Triodopsis tridentata* and *Anguispira alternata*. We do not know, however, whether Leidy diluted the contents of the seminal receptacle with water or with a saline solution, or how accurately he intended to draw the parasites he observed.

Locality: Philadelphia, Philadelphia County, Pennsylvania, USA.

Redescription: Neotype slide deposited in the United States National Museum, Washington D. C. (USNM no. 1021509), this is a smear preparation, impregnated by the protargol method, containing numerous individuals, considered to be syntypes, from the seminal receptacle of one *N. albolabris albolabris* collected near Newport, Perry County, Pennsylvania. Another slide (USNM no. 1021510) is a hematoxylin-stained preparation from the same host snail.

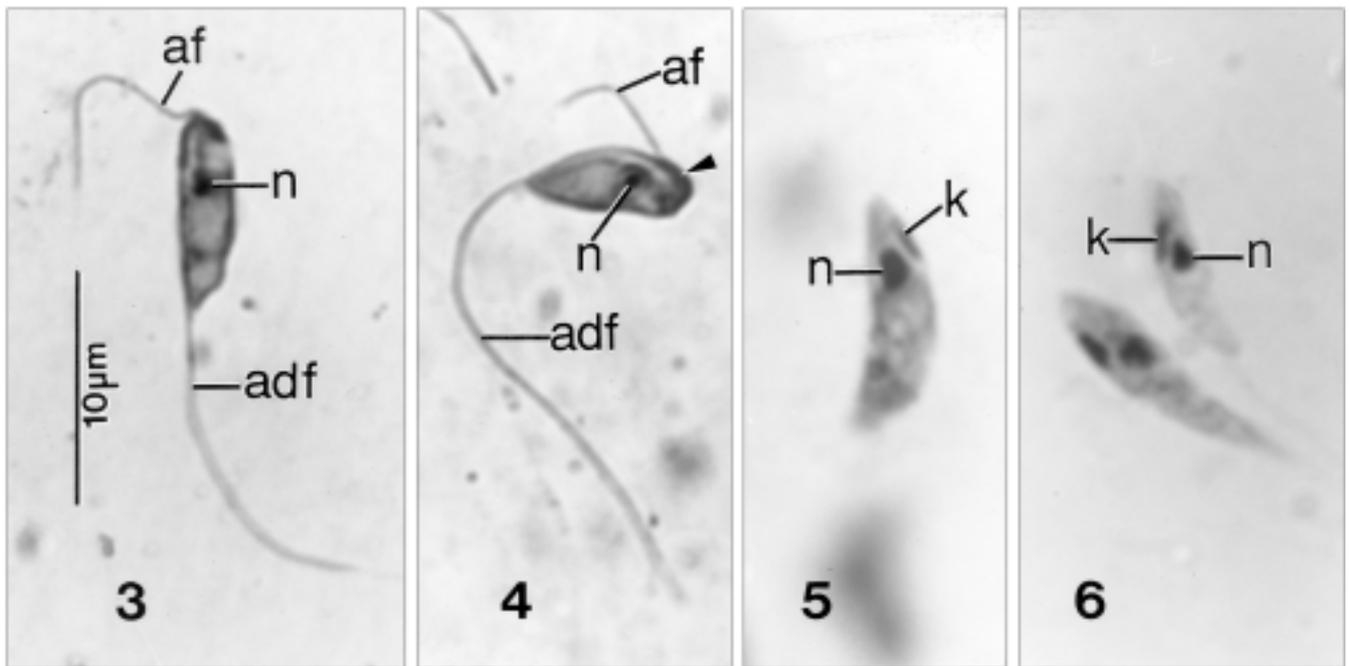
Living specimens from the type collection, selected to show extremes of size, measured from 11.25 by 1.25 μm to 27 by 2.25 μm . The body (Fig. 1) was usually 10 to 12 times as long as wide, but occasionally proportionately longer or shorter individuals were observed. The anterior end was rounded and the posterior end typically tapered to a point. Flagellates fixed in Hollande's fluid and impregnated with protargol (Figs 3, 4) are usually much shorter than living specimens and only 5 to 7 times as long as wide; furthermore, the posterior end is much like the anterior end in being rounded.

In freshly made smears, the free flagellum of medium and large specimens is usually about half to two-thirds as long as the body, rarely as long as or slightly longer than the body. It is more often directed to one side or posteriorly than anteriorly. The adherent flagellum does not become free until it reaches the posterior end of the body; the free portion is usually two-thirds to fully as long as the body. Flagellar length of small specimens was not studied extensively, because if they were products of recent divisions the lengths of the two flagella, in proportion to each other and to the length of the body, would be atypical for at least a short time.

During binary fission of the *Cryptobia* from *Helix aspersa aspersa*, only one of the two flagella of the parent, either the anterior or adherent one, is conferred upon each new individual; the complementary flagellum is neofomed and for a time is therefore much shorter than it normally is in a fully differentiated individual.) In protargol-impregnated specimens (Figs 3, 4), the proportionate lengths of the flagella are comparable to those of living specimens. The adherent flagellum, moreover, rarely becomes free until it has reached the posterior end of the body.

The nucleus (Figs 1, 3-6) is typically located anterior to the middle of the body, sometimes near the middle. In protargol preparations (Figs 3, 4), it is most commonly a vesicular structure with a central nucleolus, but sometimes the nucleus as a whole is rather darkly impregnated. Iron hematoxylin generally stains the entire nucleus rather intensely (Figs 5, 6).

The kinetoplast, as stained by hematoxylin (Figs 5, 6), is about 2 to 2.5 μm long and usually more or less rodlike, but sometimes thickest near its anterior or posterior end; its posterior portion presumably has a mitochondrion joined to it, but this has not been distinguished. Nothing unequivocally comparable to the distinctive "aciculum" seen in protargol impregnations of the *Cryptobia* from *Helix pomatia* and *H. aspersa aspersa* has been identified, but a slightly crescentic peripheral darkening



Figs 3-6. *Cryptobia heliis* Leidy, photomicrographs of specimens fixed in Hollande's fixative. **3, 4** - specimens impregnated with protargol; **5, 6** - specimens stained with iron hematoxylin. adf - adherent flagellum, af - anterior flagellum, k - kinetoplast, n - nucleus. The arrowhead in Fig. 4 indicates a darkened area that may correspond to the "aciculum" of *C. heliigenae*.

in the same part of some specimens (Fig. 4) perhaps indicates such a structure. The cytoplasm of living flagellates has small granular inclusions, but these are not conspicuous in specimens stained with hematoxylin or impregnated by the protargol method.

Flagellates in several *Neohelix solemi* collected at Chapel Hill, Orange County, North Carolina, USA ranged in size from 12 by 1.25 μm to 25 by 2.25 μm , and the appearance of living, stained, and protargol-impregnated flagellates conformed to my redescription of *C. heliis*. If there is a difference that can be detected by light microscopy, it has eluded me, and I tentatively consider the *Cryptobia* in *N. solemi* to be morphologically identical to *C. heliis* from *N. albolabris albolabris*.

Description of *Cryptobia innominata* sp. n. (Figs 2, 7-12)

Host: *Triodopsis tridentata*.

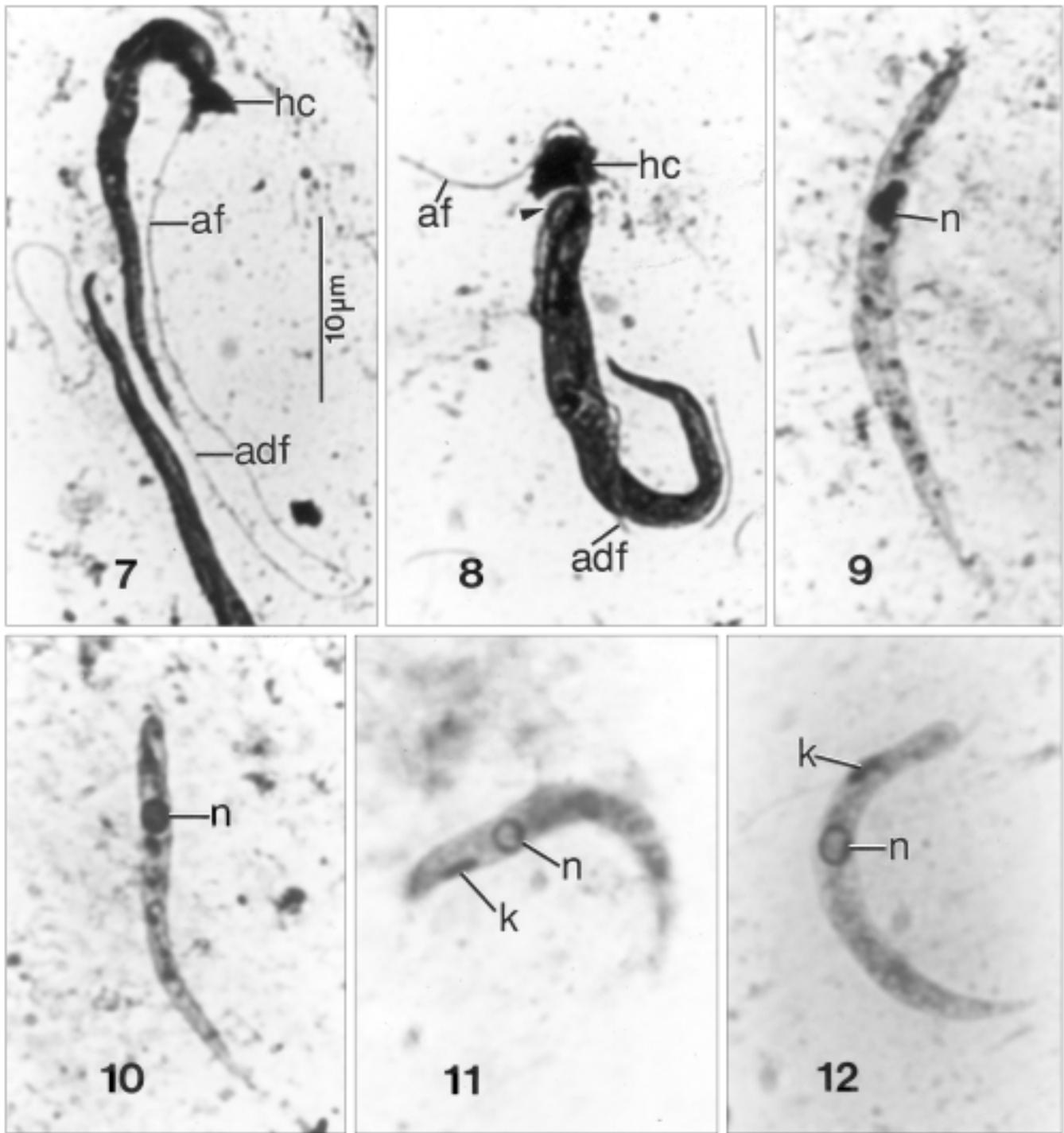
Type locality: Powder Mill Nature Reserve, Westmoreland County, Pennsylvania, USA.

Etymology: The name *innominata*, meaning "not named," alludes to the fact that this species, apparently never restudied, had been assumed to be identical with *C. heliis* from *Neohelix albolabris albolabris*.

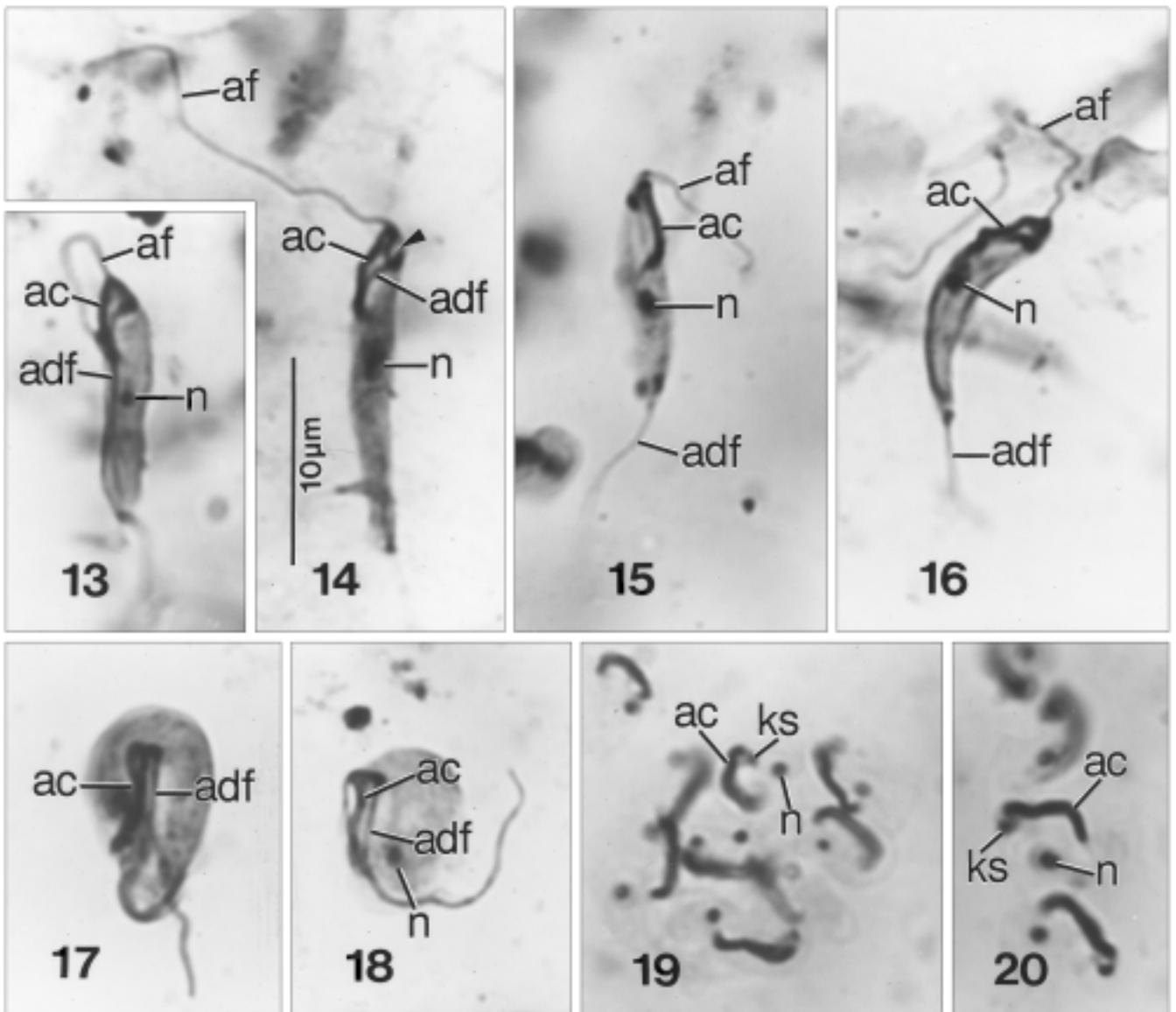
Description: Holotype slide deposited in the United States National Museum, Washington D. C. (USNM 1021511); this is a smear preparation containing numerous specimens (syntypes) impregnated by the protargol method. Another smear impregnated with protargol (USNM 1021512) and one stained with iron hematoxylin (USNM 1021513) have also been deposited.

This species, in general, is considerably larger than *C. heliis*, and its shape is typically more slender and more variable (Figs 2, 7-12). Numerous living specimens, measured at random in fresh smears from two snails, ranged in size from 22.4 by 1.35 μm to 50.5 by 2.25 μm . Vigorous serpentine movements of the body are characteristic. After fixation, specimens become proportionately shorter and wider than they were when alive.

The proportionate length of the flagella varies considerably, as is to be expected in view of the fact that one or the other is retained during division and the other is neoformed. In general, however, the anterior flagellum ranges from about one-fourth the length of the body (Fig. 8) to decidedly longer than the body (Fig. 7). In smears that have been stained or impregnated, as well as in fresh preparations, the proximal portion of the anterior



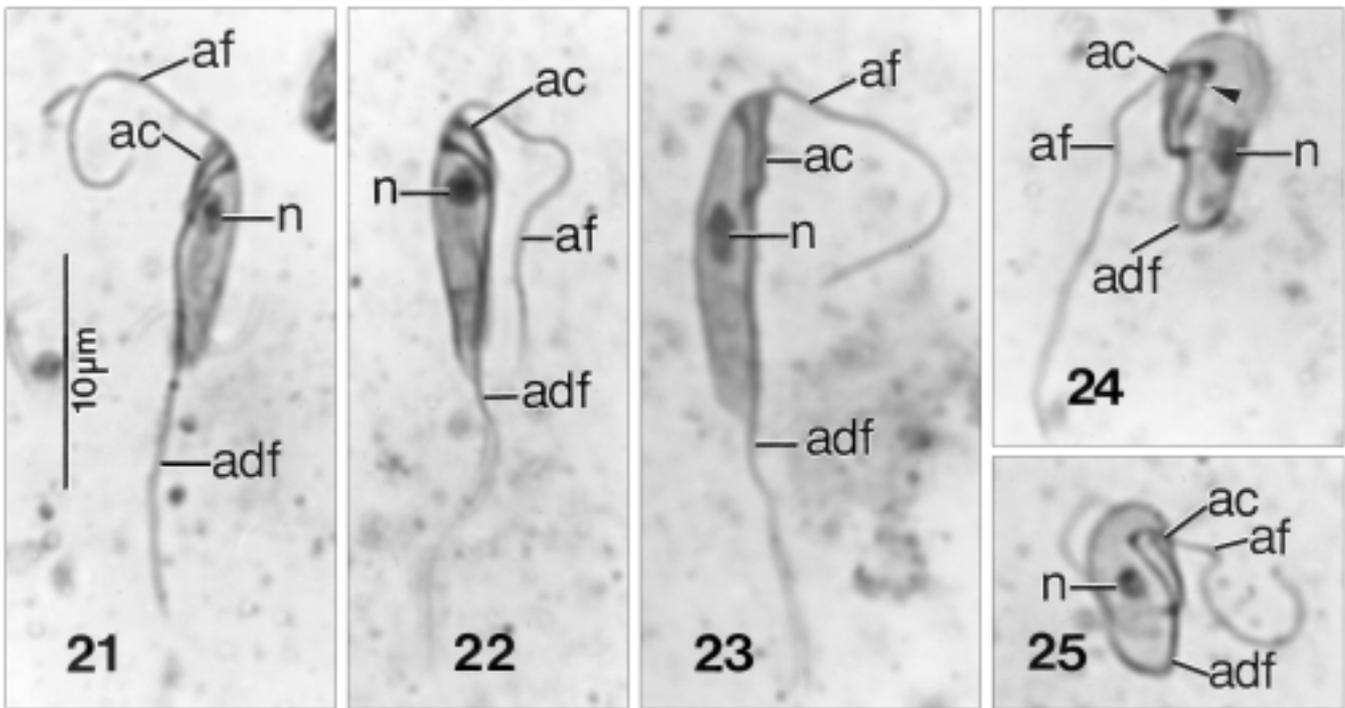
Figs 7-12. *Cryptobia innominata* sp. n., photomicrographs of specimens fixed in Hollande's fluid. **7-10** - specimens impregnated with protargol; **11, 12** - specimens stained with iron hematoxylin. adf - adherent flagellum, af - anterior flagellum, hc - cell from host tissue, k - kinetoplast, n - nucleus. The arrowhead in Fig. 8 indicates a darkened area that may correspond to the "aciculum" of *C. helicogena*.



Figs 13-20. *Cryptobia helicogena* from *Helix pomatia*, fixed in Hollande's fluid and impregnated with protargol. **13-16** - elongated individuals; **17-20** - somewhat rounded forms; **19, 20** - only the aciculum and nucleus are distinct, but the kinetosomes from which the flagella originate are so heavily impregnated that they cannot be distinguished. ac - "aciculum," adf - adherent flagellum, af - anterior flagellum, ks - kinetosomes, n - nucleus. The arrowhead in Fig.14 is directed at what appears to be a connective between the anterior portion of the "aciculum" and proximalmost part of the adherent flagellum.

flagellum is often seen to be embedded in a fragment of a cell, presumably one that was part of the epithelial lining of the seminal receptacle (Figs 7, 8). The adherent flagellum usually extends well beyond the posterior end of the body (Fig. 7). In living specimens, it typically does not separate from the body until it reaches the posterior end, but fixation often causes a considerable portion of it to become detached.

The nucleus is usually located anterior to the middle of the body. In hematoxylin-stained smears, the chromatin forms a distinct peripheral layer (Figs 11, 12); a nucleolus is rarely evident. In protargol-impregnated preparations, the contents of the nucleus are usually darkened rather uniformly (Figs 9, 10). The kinetoplast of *C. innominata* occupies a position similar to that of *C. helcis*. The cytoplasm of living specimens contains



Figs 21-24. *Cryptobia* cf. *helicogenae* from *Helix aspersa aspersa*, fixed in Hollande's fluid and impregnated with protargol. **21-23** - elongated individuals; **24, 25** - somewhat rounded forms. The arrowhead in fig. 24 is directed at what appears to be a connective between the anterior part of the "aciculum" and the proximalmost part of the adherent flagellum. ac - "aciculum," adf - adherent flagellum, af - anterior flagellum, n - nucleus.

numerous conspicuous inclusions, and these are especially distinct in lightly impregnated specimens (Figs 9, 10). Perhaps at least some of them correspond to the microbodies, food vacuoles, and symbiotic bacteria noted in other species of *Cryptobia* by Brugerolle *et al.* (1979) and Current (1980).

In protargol preparations, a few specimens (Fig. 8) exhibit a darkly impregnated area that could conceivably be comparable to the "aciculum" of the cryptobias from *Helix pomatia* and *H. aspersa aspersa*. This will be discussed later.

Names that may be applied to species of *Cryptobia* in European pulmonate snails

Now that *C. helicis* parasitizing *Neohelix albolabris albolabris* has been redefined and the flagellate observed by Leidy in *Triodopsis tridentata* has been described, it is necessary to decide what to call the cryptobias found in *Helix pomatia* and *H. aspersa aspersa*. The name *C. helicogenae* (Kühn, 1911) (described as *Trypanoplasma helicogenae*) can confidently be applied to the flagellate from *H. pomatia*,

which, as "*C. helicis*," was the object of cytological studies by Friedrich (1909), Jollos (1911), Bělař (1916, 1926), Schindera (1922), Pyne (1959, 1960), and Brugerolle *et al.* (1979), and also used in a molecular study of the kinetoplast (Lukeš *et al.* 1998, 2002; Doležel *et al.* 2000; Simpson *et al.* 2002). Because he had not studied flagellates from the hosts Leidy had examined, Kühn (1911) wisely treated genuine *C. helicis* as a *species inquirenda*. He did not designate a type specimen or a type locality for *C. helicogenae*, but stated that parasitized specimens of *H. pomatia* were collected at Gutenstein, Germany and at Budapest, Hungary.

So far as I have been able to determine, the *Cryptobia* from *H. aspersa aspersa* is morphologically identical to *C. helicogenae*. Protargol impregnations reveal the "aciculum" of *C. helicogenae* from *H. pomatia* (Figs 13-18) just as clearly as they do that of specimens from *H. aspersa aspersa* (Figs 21-25); in many specimens from both hosts, in fact, the aciculum, kinetosomal mass, and nucleus are often conspicuous when the flagella and outlines of the cells show faintly or not at all (Figs 19, 20). Another distinctive minor detail often seen in protargol

preparations of flagellates from *H. pomatia* and *H. aspersa aspersa* is what appears to be a delicate connective between the anterior portion of the aciculum and the proximalmost part of the adherent flagellum (Figs 14, 24). I am unable to reconcile this with any elements shown in the spatial reconstruction published by Brugerolle *et al.* (1979).

A neotype slide of *C. helicogenae*, bearing numerous protargol-impregnated specimens that can be considered to be syntypes, has been deposited in the United States National Museum, Washington D. C. (USNM no. 1021514), together with a second slide from the same collection (no. 1021515). The host snail was *Helix pomatia* found near České Budějovice, Czech Republic. Two slides of protargol-impregnated smears from *Helix aspersa aspersa* collected in Oakland, Alameda County, California, USA have also been deposited (USNM nos. 1021516a, 1021516b).

DISCUSSION

What I described as the “aciculum” of *C. helicogenae* from *H. aspersa aspersa* is almost certainly the bundle of microtubules that reinforce the pharynx (see Brugerolle *et al.* 1979, p. 202, Fig. 7). In his study of the *Cryptobia* from *Webbhelix multilineata*, Current (1980, p. 286, Fig. 17) showed a relatively short but similar microtubular complex. The somewhat crescentic argentophilic deposit seen in the anterior part of the body of some *C. helicis* (Fig. 4) and *C. innominata* (Fig. 8) perhaps indicates a comparable structure.

The systematics of flagellates in the genus *Cryptobia* is certain to become extremely difficult as more pulmonate snails are examined for the presence of these organisms. Living specimens from a single host cannot be characterized by much more than size, shape, and proportionate lengths of the flagella. In stained and impregnated preparations, the position and appearance of the nucleus and kinetoplast may be the only helpful features, unless there are other discrete structures, such as the “aciculum” of *C. helicogenae*.

Relatively few snails have, up to now, been examined for the presence of cryptobias. These flagellates have, however, been found in several families of stylommatophoran and basommatophoran pulmonates. Because they are transmitted from one host to another during copulation, it is probable that cryptobias in species of snails that are reproductively isolated are in some

way, morphological or molecular, distinct. The possibility that *C. helicogenae* in *H. pomatia* is slightly different from the flagellate in *H. aspersa aspersa* has already been mentioned. Because the two species of *Helix* do not mate, it is likely that their flagellates have been isolated for a long time. Even *H. aspersa aspersa* and *H. aspersa maxima*, as recognized by Chevallier (1977) are not likely to mate in nature, although there has been some success with attempts to induce copulation in captivity (Gomot-de Vaufléury and Borgo 2001).

In the literature of protozoology, it is too often assumed that parasites living in closely related hosts are identical if they appear to be similar. Future investigators dealing with cryptobias should certainly specify, in papers dealing with similar flagellates from more than one host species, which parts of a description and which figures apply to material from a particular host. Thus “*Cryptobia helicogenae* from *Helix pomatia*” will clearly designate the flagellate from the snail that has provided material for all molecular studies until now, and also for early cytological work and that of Brugerolle *et al.* (1979); “*C. cf. helicogenae* from *C. aspersa aspersa*” is the correct way to indicate the flagellate whose cytology has been studied by Kozloff (1948) and in part by Brugerolle *et al.* (1979). It is important, however, to remember that *C. helicis*, not *C. helicogenae*, is the type species of the genus. We may perhaps assume, for the time being, that the molecular attributes of *C. helicogenae* are typical of all cryptobias known to occur in pulmonate snails of North America and Europe. Nevertheless, some of these parasites may have evolved along different lines, and thorough systematic work on similarities and differences between genera requires that the type species be studied.

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John Slapcinsky sent several fully mature *Anguispira alternata* from each of two localities in Illinois, and James Atkinson provided specimens from Michigan. It was disappointing to find no flagellates in any of these, or in those I collected in Indiana. *Triodopsis fallax*, collected in North Carolina by Sue Brady and in Tennessee by

Edward Crawford, Jr. were parasitized by a *Cryptobia* somewhat similar to *C. innominata*. This material was valuable because it enabled me to experiment with procedures of staining and silver impregnation and to test various batches of protargol, and thus helped me to make more satisfactory permanent preparations of the species with which this report is primarily concerned. A period of study in the Department of Zoology, University of North Carolina, where I was able to work on *C. helicis* from *N. solemi*, was made possible by a fellowship from the John Simon Guggenheim Memorial Foundation.

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