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Three new species of *Stoecharthrum* (phylum Orthonectida)

E.N. Kozloff
Friday Harbor Laboratories, University of Washington
Friday Harbor, Washington, 98250, USA.

Résumé : *Stoecharthrum monnati* sp. nov., parasite du bivalve *Lucinoma borealis* en Bretagne, est très voisin de *S. giardi,* espèce type du genre, mais plus long et caractérisé par un plus grand nombre d'ovocytes et d'anneaux des cellules épidermiques. Chez *S. fosterae* sp. nov., hébergé par le bivalve *Mytilus trossulus* à Puget Sound, Washington, et *S. burresoni* sp. nov., parasite de l'ascidie *Ascidia callosa* à San Juan Island, Washington, les ovocytes ne sont pas en simple série comme chez *S. giardi* et *S. monnati,* mais très serrés. Par ailleurs, leurs corps sont moins minces, et la disposition des cils est différente. Donc la définition du genre *Stoecharthrum* a été légèrement modifiée.

Abstract : *Stoecharthrum monnati* sp. nov., a parasite of the bivalve *Lucinoma borealis* in Brittany, is similar to *S. giardi,* the type species of the genus, except in being longer and in having more oocytes and more rings of epidermal cells. In *Stoecharthrum fosterae* sp. nov., found in the bivalve *Mytilus trossulus* in Puget Sound, Washington, and *S. burresoni* sp. nov., found in the ascidian *Ascidia callosa* on San Juan Island, Washington, the oocytes are crowded into the axial mass instead of being arranged in a single row as they are in *S. giardi* and *S. monnati.* In addition, their bodies are proportionately less slender and their patterns of ciliation are appreciably different. The definition of the genus *Stoecharthrum* has therefore been slightly modified.

INTRODUCTION

Since Caullery and Mesnil (1899a) published the original description of *Stoecharthrum giardi*, the genus has remained monotypic. Until recently, the only other original contributions dealing with this orthonectid were also by Caullery and Mesnil (1899b, 1901) ; they illustrated the species for the first time in the 1901 paper.

The host from which Caullery and Mesnil obtained their material of *S. giardi* was the orbiniid (ariciid) polychaete *Scoloplos armiger,* collected at l'Anse Saint-Martin, Cap de la Hague, France. I have found the parasite in the same host at Roscoff and at two other localities nearby, and have added (Kozloff, 1992) some details concerning the morphology of this interesting orthonectid.

In his textbook summary of orthonectids, Caullery (1961) mentioned that a species in smears of tissue from the bivalve *Lucinoma lucinalis* (*Loripes lacteus*) collected at Roscoff seemed to be *S. giardi*. I did not find any parasitized specimens of *L. lucinalis* at Roscoff, but through the courtesy of Jean-Yves Monnat, I have been able to study a *Stoecharthrum* occurring in *Lucinoma borealis*. Although it is similar to *S. giardi*, it must be considered to be a separate species, as Monnat himself suspected. Monnat pointed out to me, furthermore, that a form of *L. borealis*, called *minor*, is difficult to distinguish from *L. lucinalis*. Because of this, and because his attempts, like my own, to find an orthonectid in genuine

L. lucinalis were unsuccessful, he suggested that the smears sent to Caullery were probably from *L. borealis*.

In this publication, I will formally describe the species from *L. borealis*, as well as two other species of *Stoecharthrum*. One of these was found in the bivalve *Mytilus trossulus* ; the other was discovered in the ascidian *Ascidia callosa*.

METHODS

Some study of live material was possible in the case of the orthonectids from *Mytilus trossulus* and *Ascidia callosa*, but my observations on the species from *Lucinoma borealis* were limited to preparations sent to me by Jean-Yves Monnat. Three methods were used in making permanent mounts. One of these was impregnation with silver nitrate, which demonstrates the boundaries of the epidermal cells. In this technique, fresh smears of tissue from parasitized hosts were dropped face down on a 2 % aqueous solution of silver nitrate, then exposed to bright north light while still in the silver nitrate solution or after being transferred to distilled water. Impregnation by the Protargol method, to show the arrangement of kinetosomes of cilia and some other structures, followed fixation of smears in Bouin's fluid or Champy's fluid. Staining with iron hematoxylin was carried out with material fixed in Bouin's fluid.

All measurements are based on specimens impregnated with silver nitrate. Although dehydration in alcohol leads to some shrinkage of orthonectids prepared by this method, the extent of shrinkage is slight.

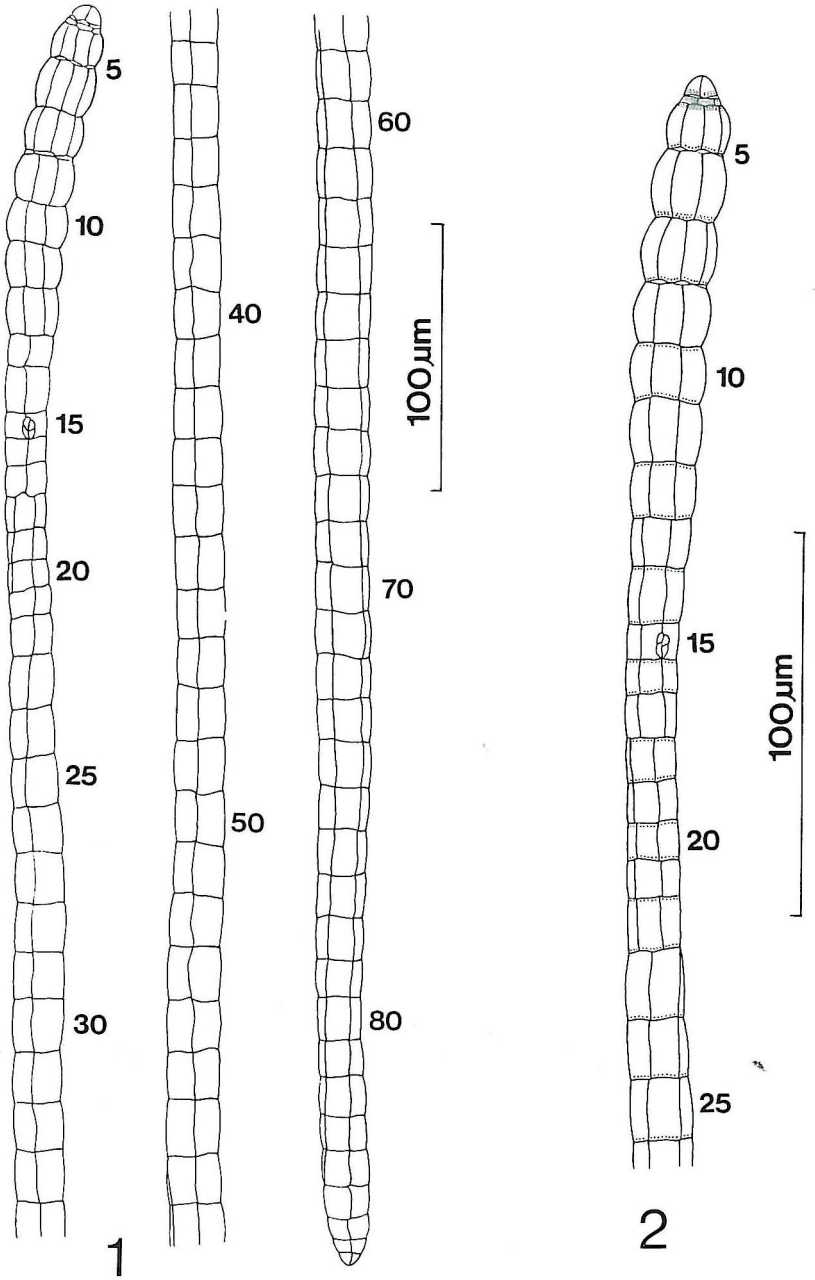
DESCRIPTION OF SPECIES

Stoecharthrum monnati sp. nov. (Figs 1-5)

This species is named for Jean-Yves Monnat, who examined 2 169 *Lucinoma borealis* collected at An Deleg (Rade de Brest), Kastell-Meur/Plougrescant (Côtes d'Armor), and Lilia/Plouguerneau, Brignogan, and Drezol/Santec (all Finistère-Nord). He found 60 (2.8 %) to contain adult orthonectids. In *L. borealis* from An Deleg, however, Monnat searched specifically for early stages of development as well as for adults, and determined that 11 of the 224 bivalves (4.9 %) had the *Stoecharthrum*.

Monnat observed that the host tissues most commonly and most intensely parasitized were those of the ctenidia, but the orthonectid was also noted in the gonad, digestive gland, and pericardial region ; in one case, the pedal ganglion was involved. In most of the smears sent to me, there are many mature specimens, as well as some immature individuals that have helped me understand the morphology of this species.

Complete mature specimens range in length from 1 490 to 1 625 µm. At the level of epidermal rings 9-12, and sometimes near the middle of the body, the width attains 24 µm.

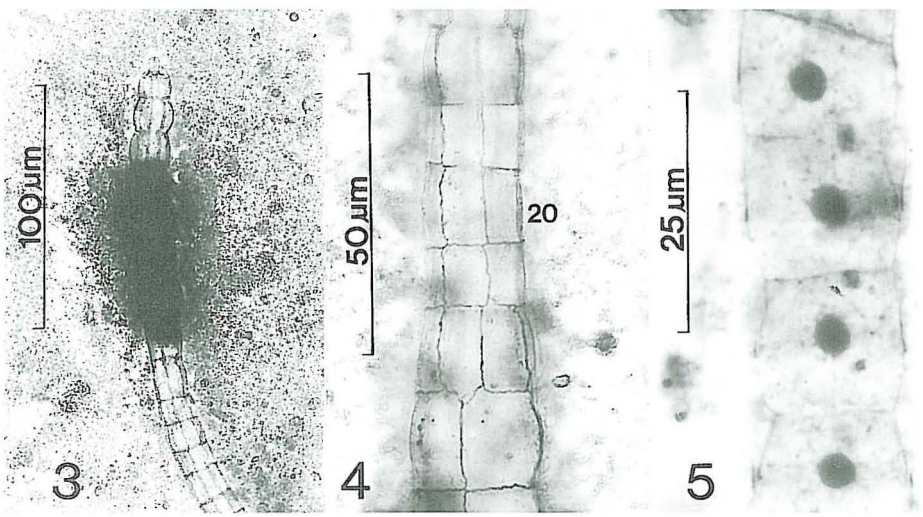


Figs. 1-2 : *Stoecharthrum monnati* sp. nov. 1. Complete specimen, showing boundaries of epidermal cells ; silver nitrate impregnation. 2. Anterior portion ; composite drawing, based on specimens impregnated with silver nitrate to show cell boundaries, and also on specimens impregnated by the Protargol method to show the arrangement of kinetosomes.

Favorably impregnated specimens in my material (Figs. 1-4) have from 87 to 96 rings of epidermal cells. Although the number greatly exceeds that in *S. giardi*, the arrangement and characteristics of the epidermal cells is the same in both species. Furthermore, the pattern of ciliation (Fig. 2) is essentially identical. As in *S. giardi*, the apical cells of ring 1 have two transverse rows of cilia near their posterior borders, and there are also two rows on the small exposed surfaces of the cells of ring 3, near the anterior borders of ring 4, and near the posterior borders of ring 6. Rings 2, 5 and 8, whose exposed surfaces are very small, lack cilia ; rings 9, 11, 13, 15, 17, 19, and 21, whose surfaces are comparatively large, also lack cilia ; rings 10, 12, 14, 16, 18, 20, and 22 have cilia near both their anterior and posterior borders ; ring 7 and all rings from 23 to the posterior end of the body have cilia only near their posterior borders.

The epidermal cells of rings 9-12, like those of *S. giardi*, are filled with refractile granules that remain evident in silver nitrate preparations. These cells are conspicuously blackened by impregnation (Fig. 3), presumably because of a reaction between the granules and the silver nitrate. The blackening, in fact, usually makes it difficult to see the boundaries of the epidermal cells in this region. Immature specimens, with fewer inclusions and therefore less blackening, have been useful in confirming the arrangement of epidermal cells in rings 9-12.

The genital pore, surrounded by four small nonciliated cells that collectively form an oval, is located in ring 15 (Figs. 1, 2). When sperm or precursors of sperm can be seen, they occupy an area up to 40 µm long in the region of the genital pore. In my complete speci-



Figs. 3-5 : *Stoecharthrum monnati* sp. nov.; photomicrographs. 3. Anterior portion of a specimen impregnated with silver nitrate, showing the blackening of granule-containing cells of rings 9-12. 4. Boundaries of epidermal cells of rings 18-23 ; silver nitrate impregnation. 5. Arrangement of oocytes ; Protargol impregnation.

mens, there are 62-65 oocytes, and these are arranged in a single row (Fig. 5), as in *S. giardi*. The anterior edge of the first oocyte is usually a few epidermal rings behind the level of the genital pore.

The holotype, impregnated by the silver nitrate method, has been deposited in the United States National Parasite Collection, Beltsville, Maryland (no. 82833). Two paratypes, one impregnated with silver nitrate (no. 82834), the other impregnated by the Protargol method (no. 82835), have also been deposited. All are from *Lucinoma borealis* collected by Jean-Yves Monnat at An Deleg (Rade de Brest), France. The smears containing the type specimens have numerous other individuals of *S. monnati*.

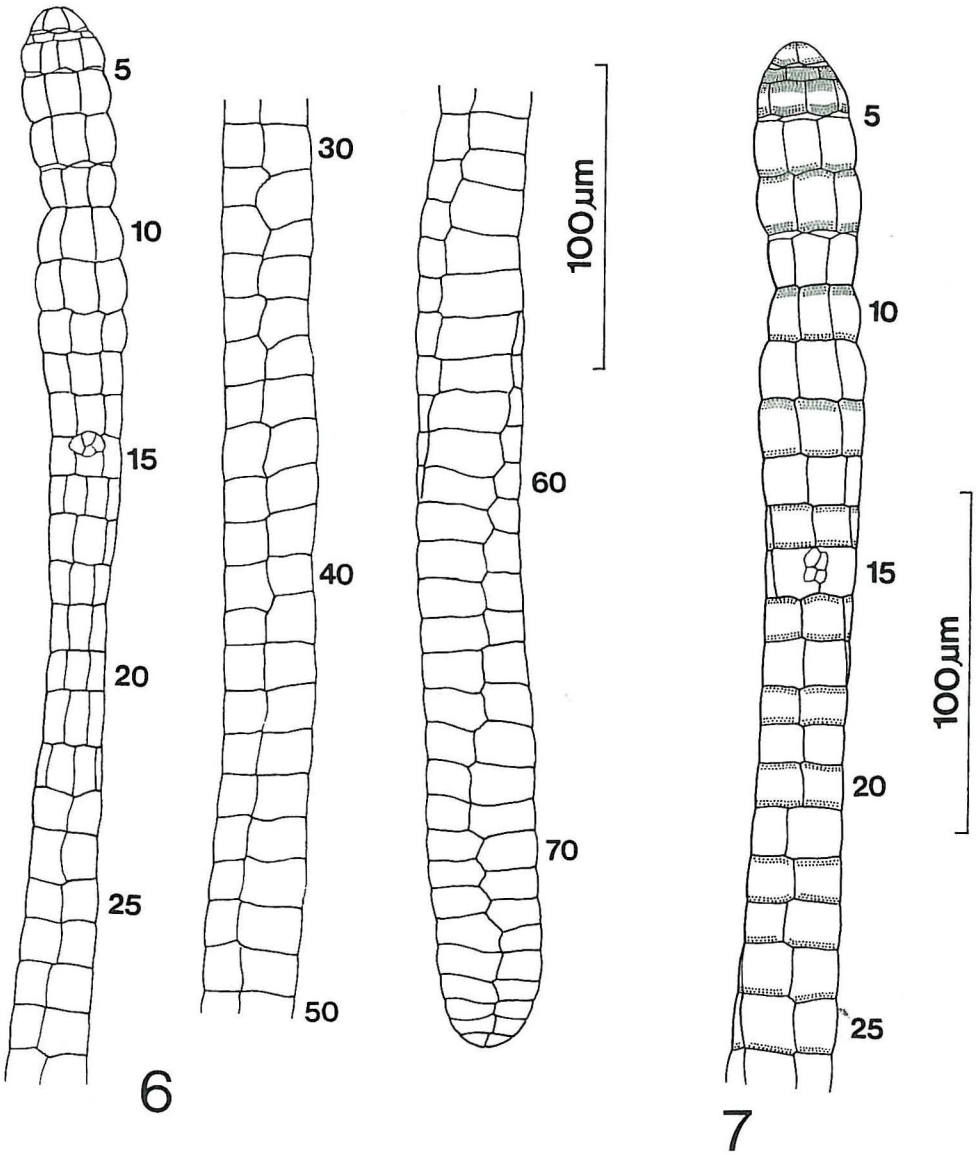
Stoecharthrum monnati differs from *S. giardi* in three major respects. It is considerably longer (up to 1 625 μm instead of 800 μm), has more rings of epidermal cells (87-96 instead of 65-69), and has more oocytes (62-65 instead of 38-42). I should mention, however, that I have not observed sperm or prospective sperm between oocytes, alongside oocytes, or in the region behind the last oocyte of *S. monnati*. Any or all of these arrangements are commonly observed in *S. giardi*, and perhaps sometimes occur in *S. monnati*.

Because the orthonectid parasites of *L. borealis* and *S. armiger* are so similar, it could be argued that we are dealing with just one species, which reaches a longer length in its bivalve host than it does in its polychaete host. If this were true, I would expect some intergradation, not only in body length, but also in the number of rings of epidermal cells and in the number of oocytes. The fact that specimens from *L. borealis* and those from *S. armiger* are so well separated with respect to these characters persuades me that *S. monnati* and *S. giardi* are distinct species.

Stoecharthrum fosterae sp. nov. (Figs 6-13)

This orthonectid was found in 1981 in specimens of what was, at that time, considered to be *Mytilus edulis*. The mussels, grown on rafts in Totten Inlet, in southern Puget Sound, Washington, were collected by personnel of Kamilche Sea Farms and given to Carolyn Foster for examination. Before contacting me, she had carefully studied the parasite and had concluded that it was an orthonectid similar to *S. giardi*. She provided me with the mussels from which I obtained all of my material, and collaborated with me in making permanent preparations. She also published a brief report (Foster, 1982) on the occurrence of the parasite. It gives me great pleasure to name this species for her.

The native mussel in Puget Sound has long been considered to be *Mytilus edulis* L., 1758. Although Lamy (1936) had concluded that mussels of the *Mytilus edulis* complex from the Pacific coast of North America should be referred to *M. trossulus* Gould, 1850, Soot-Ryen (1955) considered *M. trossulus* to be just one of several subspecies of *M. edulis*. McDonald and Koehn (1988), depending mostly on results obtained by electrophoresis, decided that mussels from Alaska, Oregon, northern California, the Baltic Sea, and the Pacific coast of Siberia, as well as some mussels from eastern Canada, are indeed sufficiently distinct from the strictly Atlantic *M. edulis* to warrant their being called *M. trossulus*. In papers by Koehn (1991), McDonald, Seed, and Koehn (1991), Koehn



Figs. 6-7 : *Stoecharthrum fosterae* sp. nov. 6. Complete specimen, showing boundaries of epidermal cells ; silver nitrate impregnation. 7. Anterior portion ; composite drawing, based on specimens impregnated with silver nitrate to show cell boundaries, and also on specimens impregnated by the Protargol method to show the arrangement of kinetosomes.

(1992), and Seed (1992), the systematics and geographic distribution of the *M. edulis* complex are discussed thoroughly. For our purposes, it is sufficient to say that native mussels in Puget Sound, including those from which the orthonectid parasite was obtained, may be identified as *M. trossulus*.

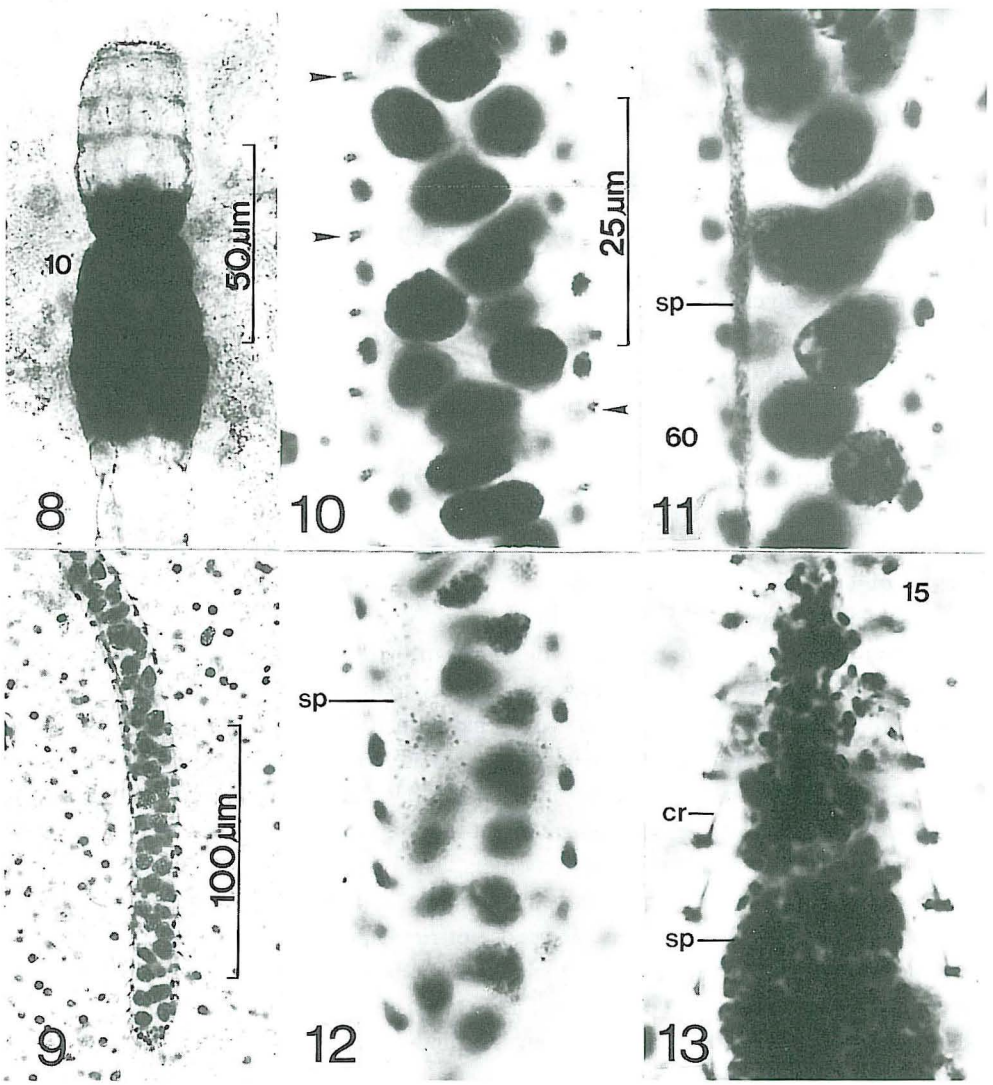
Mytilus galloprovincialis Lamarck, 1819, which occurs in California, eastern Asia, western Australia, the Mediterranean Sea, and western Europe, is now being grown for food in Puget Sound, but the commercial stocks are derived from hatchery-reared young. Whether *M. galloprovincialis* has established itself in the region is uncertain. In any case, it is not known to have been cultivated in Puget Sound as early as 1981.

Of 73 mussels examined by Foster during July, 1981, 15 % were parasitized. In some hosts, the orthonectid was limited to the mantle and gonads, but in others it was present in the digestive gland, adductor muscle, and ctenidia. I examined 12 specimens from the same source and found two to be parasitized.

Mature specimens of *S. fosterae*, in smears impregnated with silver nitrate, range in length from 725 to 970 µm. The greatest width--34 µm--is usually in the posterior half, sometimes close to the posterior end. The body is rather conspicuously narrowed in the second one-eighth of its length.

Complete specimens (Fig. 6) have from 67 to 78 rings of epidermal cells. In certain details, the pattern of ciliation (Fig. 7) differs from that of *S. giardi* and *S. monnati*. The cells of ring 1 have two transverse rows of cilia close to their apices and two rows near their posterior borders. The cells of ring 2, which have small exposed surfaces, lack cilia. The cells of ring 3, with surfaces approximately twice as large as those of ring 2, are almost completely covered with cilia. The kinetosomes of these are arranged in about four irregular rows. In each cell of ring 4, there are about four similar rows near the anterior borders and about three rows near the posterior borders. The cells of ring 5 are like those of ring 2 in having small surface areas and in being nonciliated. The cells of ring 6 have two rows of cilia near their posterior borders, and those of ring 7 have two rows of cilia near their anterior borders and three rows near their posterior borders. Ring 8, consisting of small nonciliated cells, and ring 9, consisting of large cells, are nonciliated. Rings 10, 12, 14, 16, 18, 20, and 22 have cilia near both their anterior and posterior borders ; in rings 10 and 12, the ones near the anterior borders of the cells are in about three rows, but elsewhere they are in double rows. From ring 23 onward, the cells of each ring have a double row of cilia near their posterior borders. The double nature of the rows is most clearly seen when specimens in which the kinetosomes are sharply impregnated are viewed in optical section (Fig. 10). Rings 11, 13, 15, 17, 19, and 21 lack cilia.

The genital pore, in the midst of several small cells--usually four, sometimes six--is located in ring 15 (Fig. 6). There is often a mass of sperm or precursors of sperm in the vicinity of the genital pore, and in many specimens sperm are found at more posterior levels (Figs. 11, 12). In a few specimens, what I believe to be precursors of sperm occupy most of the axial mass posterior to the level of the genital pore (Fig. 13). Such individuals appear to be purely male, but I have not seen any that were filled with mature sperm. The oocytes,



Figs. 8-13 : *Stoecharthrum fosterae* sp. nov. Figs 11-13 are to the same scale as Fig. 10. 8. Anterior portion of a specimen impregnated with silver nitrate, showing the blackening of granule-containing cells of rings 9-13. 9. Posterior portion, showing arrangement of oocyte nuclei ; Protargol impregnation. 10. Optical section of a small portion, showing oocyte nuclei and double rows of kinetosomes (those that are most distinct are indicated by arrowheads) ; Protargol impregnation. 11. Sperm (sp) alongside oocytes ; Protargol impregnation. 12. Sperm (sp) and oocytes near the posterior end ; Protargol impregnation. 13. Precursors of sperm (sp) in a specimen that appears to have no oocytes ; cr, ciliary rootlet ; Protargol impregnation.

unlike those of *S. giardi* and *S. monnati*, are not in a single row (Figs 9-12). Their crowded arrangement, in fact, resembles that in females of species of *Intoshia*. In 15 complete specimens in which I could count the oocyte nuclei, the number ranged from 131 to 145.

The epidermal cells of rings 9-13 contain conspicuous granules similar to those in the comparable portion of the body of *S. giardi* and *S. monnati*. The granules are dissolved by acid fixatives. In silver nitrate preparations, however, they persist, and the cells containing them are deeply blackened (Fig. 8). A characteristic feature of the body of *S. fosterae* is an obvious constriction at the junction of rings 9 and 10.

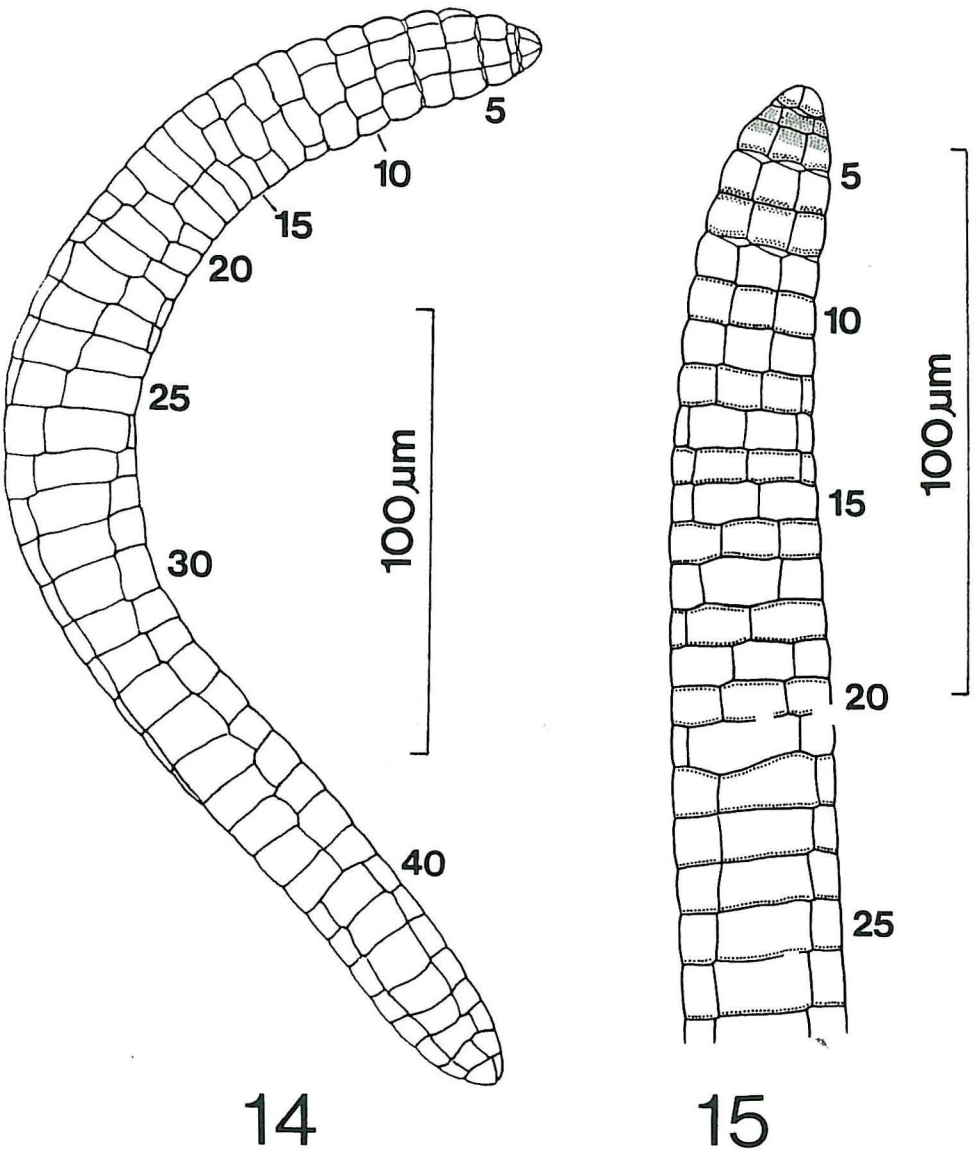
The holotype, impregnated by the silver nitrate method, has been deposited in the United States National Parasite Collection (no. 82836). Two paratypes, one impregnated by the silver nitrate method (no. 82837), the other impregnated by the Protargol method (no. 82838), have also been deposited. All of the types are from *Mytilus trossulus* collected in Totten Inlet, Puget Sound, Washington. There are many other specimens, mostly fragments, in the smears containing the type specimens.

Stoecharthrum burresoni sp. nov. (Figs 14-18)

This species is named for Eugene Burreson, who recognized it as an orthonectid when he discovered it, in 1968, in *Ascidia callosa* collected on floating docks at Roche Harbor, San Juan Island, Washington. In the three hosts I examined, the orthonectid was present almost throughout the visceral mass, but perhaps some tissues were affected more than others. Attempts to find additional parasitized specimens have not been successful.

The length of silver nitrate-impregnated specimens of *S. burresoni* ranges from 300 to 365 μm. In undistorted specimens that are at or close to the maximum length, the greatest width, near the middle of the body, is 39 μm, but most specimens are no wider than 36 μm.

The number of rings of epidermal cells ranges from 40 to 46, and their general arrangement (Fig. 14) is comparable to that of *S. fosterae*. Because my best Protargol preparations of *S. burresoni* are not especially good for demonstrating kinetosomes, I cannot be sure that I have worked out the pattern of their distribution exactly. My uncertainty is due in part to the fact that certain assemblages of kinetosomes are neither in a single transverse row nor in two clearly separate rows ; they appear to be more or less interdigitated in such a way that they form incompletely double rows. Nevertheless, the pattern of ciliation (Fig. 15) conforms, in general, to that of other species of *Stoecharthrum*. The apical cells of ring 1 have two rows of cilia near their posterior borders, but the small cells of ring 2 lack cilia. The cells of ring 3, whose surfaces are appreciably larger than those of ring 2, are more or less completely covered by three or four rows of cilia. A similar arrangement of cilia is characteristic of the anterior portions of the cells of ring 4, which also have a single, double, or incompletely double row of cilia near their posterior borders. Ring 5, consisting of small cells, is not ciliated. In ring 6, the posterior margins of the cells appear to have a double or incompletely double row, whereas in ring 7 there is a single row near the anterior borders of the cells and a double or incompletely double row near the posterior borders. The small



Figs. 14-15 : *Stoecharthrum burresoni* sp. nov. 14. Complete specimen, showing boundaries of epidermal cells ; silver nitrate impregnation. 15. Anterior portion ; composite drawing, based on specimens impregnated with silver nitrate to show cell boundaries, and also on specimens impregnated by the Protargol method to show the arrangement of kinetosomes.

cells of ring 8 are not ciliated. Rings 9, 11, 13, 15, 17, 19, and 21 lack cilia. Rings 10, 12, 14, 16, 18, 20, and 22 have what I interpret to be a single transverse row of cilia near both their anterior and posterior borders, but the cells from ring 23 onward have cilia only near their posterior borders.

In ring 15 of some specimens, a rosette of four small cells indicates the presence of a genital pore. These cells are rarely seen, however, and I do not think their apparent absence can be explained simply on the basis of the way the specimens are oriented in the smears. My silver nitrate preparations contain hundreds of sharply impregnated specimens, yet I have seen cells of the type normally associated with a genital pore in only a few of them. The oocytes (Fig. 17) occupy the axial mass from the level of the genital pore to near the posterior tip of the body. They are arranged in much the same way as in *S. fosterae*. In 15 specimens in which I counted the oocyte nuclei, the number ranged from 67 to 81. A mass of sperm, or precursors of sperm, is often present in the region adjacent to the genital pore, and sperm may also occur at more posterior levels (Fig. 17). In a few specimens in my preparations, the entire genital portion of the axial mass is occupied by precursors of sperm (Fig. 18) ; I do not know, however, if sperm mature in such apparently purely male individuals.

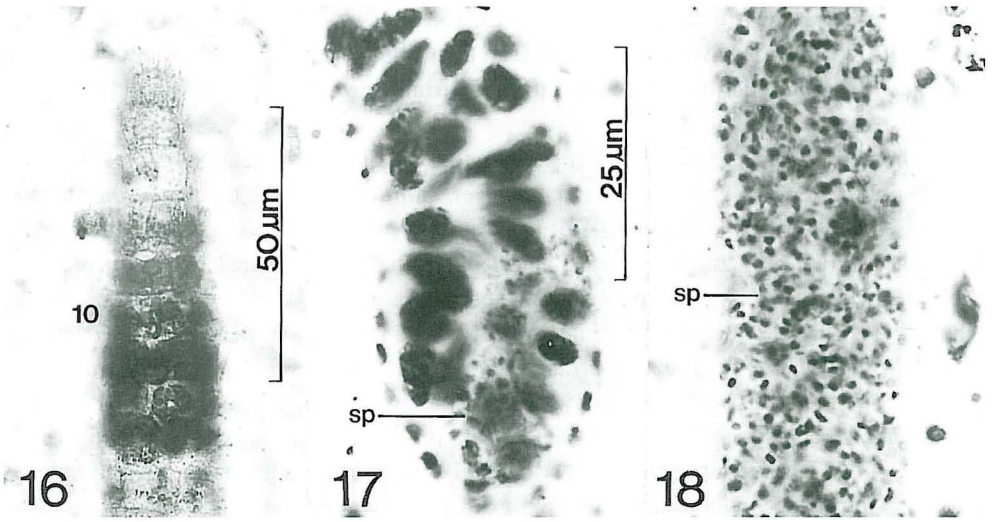
The epidermal cells of rings 7 and 9-13 (Fig. 16) contain granules of the same type as are characteristic of the corresponding portion of the body of *S. giardi*, *S. monnati*, and *S. fosterae*. While the exposed surfaces of these cells may bulge slightly, there is not an obvious constriction such as is typical of the junction of rings 9 and 10 in *S. fosterae.*

The holotype, impregnated by the silver nitrate method, has been deposited in the United States National Parasite Collection (no. 82839). Two paratypes, one impregnated with silver nitrate (no. 82840), the other impregnated by the Protargol method (no. 82841) have also been deposited. All are from *Ascidia callosa* collected at Roche Harbor, San Juan Island, Washington. The smears containing the type specimens have numerous other individuals of *S. burresoni.*

With respect to general form, pattern of ciliation, and arrangement of oocytes, *Stoecharthrum burresoni* is more nearly similar to *S. fosterae* than to either *S. giardi* or *S. monnati*. It differs from *S. fosterae*, however, in being shorter, having fewer rings of epidermal cells and fewer oocytes. Furthermore, I have not found cilia on the apical portions of the cells of ring 1 of *S. burresoni.*

EMENDATION OF THE GENUS *STOECHARTHRUM*

In my comparative study of the genera of orthonectids (Kozloff, 1992), *Stoecharthrum* was defined on the basis of extremely elongated body shape, pattern of ciliation, hermaphroditism, and arrangement of oocytes in a single row. Although *S. monnati* agrees perfectly with the diagnosis, *S. fosterae* and *S. burresoni* do not. *Stoecharthrum burresoni* is not especially elongated, and neither *S. burresoni* nor *S. fosterae* has oocytes in a single



Figs. 16-18 : *Stoecharthrum burresoni* sp. nov. Fig. 18 is to the same scale as Fig. 17. 16. Anterior portion of a specimen impregnated with silver nitrate, showing the blackening of granule-containing epidermal cells of rings 7 and 9-13. 17. Oocytes and precursors of sperm (sp) at posterior end ; Protargol impregnation. 18. Precursors of sperm (sp) in a specimen that has no oocytes ; Protargol impregnation.

row. In other respects, however, these species conform to the characters of the genus. It seems best, therefore, to remove length as a qualification, and to state that the oocytes may be arranged either in a single row, as they are in *S. giardi* and *S. monnati*, or crowded into the axial mass, as they are in *S. fosterae* and *S. burresoni*.

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