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THE HYPERPARASITE, *UROSPORIDIUM SPISULI* SP. N. (HAPLOSPOREA), AND ITS EFFECTS ON THE SURF CLAM INDUSTRY*

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ABSTRACT: Surf clams (*Spisula solidissima*) from along the Virginia and North Carolina coasts are infected with an immature anisakid nematode worm which does not cause economic concern until infected with the protozoan hyperparasite, *Urosporidium spisuli* sp. n. When the protozoan sporulates the spores give the worm a brownish-black appearance, thus making it readily visible in clam tissues. Light and electron microscope studies of the spores reveal characteristics which differentiate the protozoan from other species of *Urosporidium*. All spores are killed after 5 to 30 min at 100 C; therefore, the consumer would not ingest live spores when eating commercially prepared clam meat.

Surf clams (*Spisula solidissima*) have become an important species in the United States seafood industry. In 1973 the exvessel or landing value of the surf clam industry was valued at \$9.8 M and the processed value reached a record level of about \$38 M (Anonymous, 1974). It was therefore of considerable economic concern when, in the last few years, dark brown worms were noted in the foot and adductor muscles of large numbers of S. *solidissima* from Virginia waters (Fig. 1). Samples of clams from the Atlantic Ocean in the vicinities of Oregon Inlet, N. C., False Cape, Va., and the Chesapeake Light off the Virginia Capes were found to be 16% infected with nematodes containing spores of *U. spisuli* ($N = 894$). Sightings of the brown worms resulted in clams being withheld from the commercial trade in Maryland.

Subsequent studies in this laboratory have revealed that the worms (1) were parasites of the clams prior to recognition of the commercial problem and (2) are immature anisakid nematodes similar to *Paranisakiopsis pectinis* (Cobb 1930) Mosgovoy 1951, not previously reported from S. *solidissima*. The present systematic position of the worm is uncertain, because mature individuals have not been found.

The worms were not previously noticed or reported by workers in the industry, probably because they are normally similar in color and density to the host tissues. We have determined that the nematodes become dark brown

when a haplosporidan hyperparasite, found in the pseudocoel, sporulates, forming large masses of golden brown spores contained in vermiform sporocysts (Fig. 2). The parasite has been determined to be a new species of *Urosporidium*.

Sections of *U. spisuli* and whole mounts of spores were prepared for electron microscopy using the techniques described by Perkins (1971).

DESCRIPTION

Urosporidium spisuli sp. n.

(Figs. 2–5)

Host: An immature anisakid nematode similar to *Paranisakiopsis pectinis* (Cobb 1930) Mosgovoy 1951, which parasitizes the surf clam, *Spisula solidissima*.

Locality: Vicinity Chesapeake Light, off Cape Henry, Virginia, N. Atlantic Ocean.

Habitat: Pseudocoel.

Sporulation occurs in pseudocoel to yield unbranched and branched vermiform sporocysts up to 0.5 mm long. Living spores (volume delimited by wall) ovoid, range from 3.8 to 6.9 μm by 3.0 to 5.9 μm [$N = 100$; $\bar{x} \pm (S_{\bar{x}})$ ($t_{0.05}$) = 4.9 ± 0.05 and 4.4 ± 0.04]. Epispore cytoplasm extended centrifugally into 2 or 3 slender extensions about 7 to 14 μm long. Each extension is membrane-limited and contains 1 to 3 tubes, each consisting of an electron-dense cortex surrounding a lumen. Sheets of striated material with periodicities of 15 to 17 nm are attached to the spore wall and extend throughout the epispore cytoplasm in a labyrinthuline complex. Anterior end of spore wall marked by a circular pore region which is occluded by a flap of wall material. No "spherule" or Golgi apparatus present in the spores. A 2- to 3-μm-long bundle of fibers each measuring 16 to 20 nm in diameter is found in the spore cytoplasm.
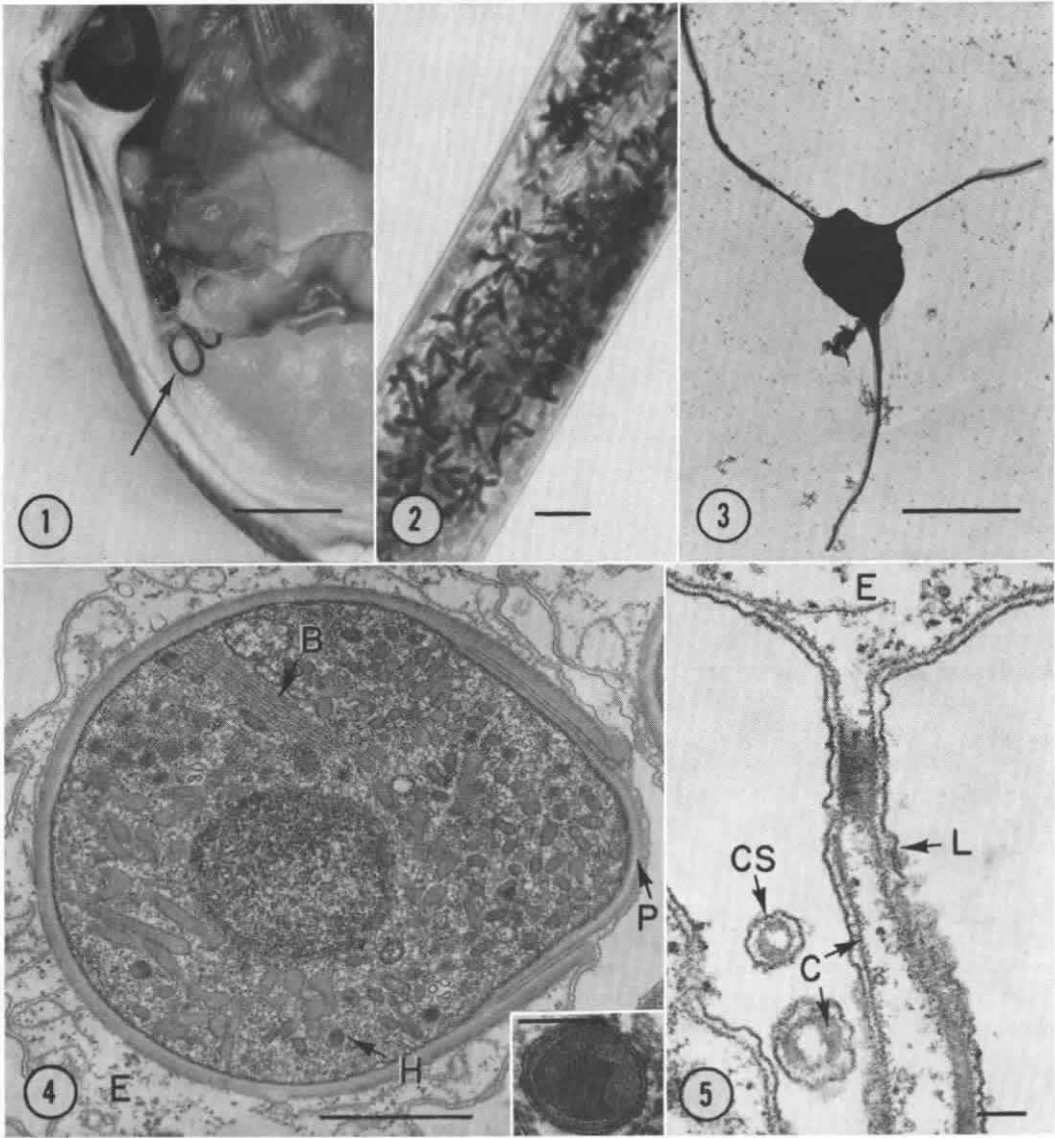
FIGURES 1–5. *Urosporidium spisuli* sp. n. parasitic in a nematode parasite of the surf clam, *Spisula solidissima*. **1.** Clam showing hyperparasitized nematode (arrow) embedded between adductor and foot retractor muscles. Bar = 10 mm. **2.** Hyperparasitized nematode containing vermiform sporocysts of *Urosporidium spisuli*. Bar = 0.5 mm. **3.** Whole mount of *U. spisuli* spore showing three appendages. Bar = 5 μm. **4.** Spore of *U. spisuli* showing pore region (P), haplosporosome (H), epispore cytoplasm (E), bundle of microtoubules (B). Bar = 1 μm. Inset: spore haplosporosome. Note delimiting unit membrane. Bar = 0.1 μm. **5.** Appendages of spore showing electron-dense core (C) in longitudinal (L) and cross-sectional orientation (CS). Epispore cytoplasm (E). Bar = 0.1 μm.

Remarks

Although it is uncertain how spores are delimited, the sporulation sequence is otherwise essentially the same as previously described (Perkins, 1971; Ormières et al., 1973).

Spores are uninucleate and have a cup-shaped ovoid wall consisting of two primary layers, the inner one being thinner and more electron-dense than the outer (Fig. 4). At the anterior end there is a circular pore delimited by a ridge

which consists of localized thickening of the wall. Within the ridge the wall is thinner than the main spore case, thus forming a circular depression or pore region as seen in other species of *Urosporidium* (Perkins, 1971; Ormières et al., 1973). The thin portion of wall material extends under the main spore wall and is attached to the overlying wall except in one area so that when the spore protoplast emerges the thin wall is turned outward through the pore.

The spore cytoplasm contains membrane-limited, electron-dense bodies, termed haplosporosomes (Perkins, 1971). They are spheroidal or oblate inclusions 85 to 149 nm along shortest axis ($N = 50$) with a bipartite substructure (Fig. 4). An inner electron-light zone separates the two electron-dense portions. Haplosporosomes have been found in all but one species of *Minchinia* and one species of *Haplosporidium* thus far examined by electron microscopy (Perkins, 1968, 1969, 1971, 1975; Ormières et al., 1973) and appear to be found only in the Haplosporea. The function of the organelles is unknown.

Membrane-bound vesicles and cisternae containing granular material of lower electron density than the haplosporosomes are also found scattered throughout the cytoplasm (Fig. 4) rather than being organized into an anastomosing complex of cisternae as in *U. crescens* and *U. jiroveci*. The cisternae of *U. spisuli* range from spherical to tubular (branched or straight) units with cross-sectional diameters of 80 to 230 nm ($N = 20$). Tubular units may be as long as 2.5 μm.

Haplosporosomes appear to be formed within or from the cisternae as evidenced by intermediate stages. The sequence is not in reverse order as seen by the increase in numbers of haplosporosomes and decrease in the cisternae as spores mature. *U. crescens* appears to utilize the same morphogenetic sequence (Perkins, 1971).

Other organelles of the spore consist of a single nucleus and one or two tubulovesicular mitochondria. The ground cytoplasm contains a small number of membrane-free ribosomes and a limited amount of endoplasmic reticulum. One or two Golgi apparatuses are present in immature spores, but have not been seen in mature ones. Mature and immature spores

contain a 2.0- to 3.0-μm-long bundle of microfibers, each microfiber being 16 to 20 nm in diameter and extending about ⅛ to ¾ of the length of the bundle (Fig. 4). Cross sections of the microfibers reveal a hexagonal packing involving up to about 300 microfibers.

Surrounding the developing spore and outside the wall is a layer of anucleate cytoplasm, the epispore cytoplasm. Extending from the layer are two or three rodlike, membrane-limited appendages which reach a length of 7 to 14 μm (Fig. 3). Each rod consists of one to three cores, each of which, in turn, consists of a tubular structure which has an electron-dense cortex and electron-light center (Fig. 5). The cores arise from sheets of striated material with periodicities of 15 to 17 nm. The sheets are attached to the spore wall and form a labyrinthine complex in the epispore cytoplasm.

DISCUSSION

U. spisuli is considered to be a member of the genus because: (1) the spore wall and sporoplasm closely resemble that of *U. fuliginosum* (Caullery and Mesnil, 1905), *U. crescens* (Perkins, 1971), and *U. jiroveci* (Ormières et al., 1973); and (2) epispore cytoplasm is extended into tapering structures reinforced by cytoplasmic fibrils as observed by Perkins (1971) and Ormières et al. (1973). Sporulation cell stages are consistent with those of other haplosporidans in which a sporont, then sporocyst, is formed.

U. spisuli is described as a new species, primarily because it is the first description of a species of *Urosporidium* parasitizing a nematode. The most similar species is *U. pelseneeri* (Caullery and Chappellier 1906) Dollfus 1925 found in the trematode *Cercaria pectinata* which parasitizes the clam *Donax vittatus* along the Atlantic coast of France. *U. pelseneeri* is the only other species of *Urosporidium* which normally forms two or three epispore extensions (Dollfus, 1925; Sprague, 1970). Extensions of *U. pelseneeri* are about 10 to 11 μm (Dollfus, 1925) while those of *U. spisuli* are 7 to 14 μm. Spore diameters are within the same range as *U. spisuli*. Caullery and Chappellier (1906) recorded the diameter as being 4.5 to 5 μm and Dollfus (1925) recorded 5 to 5.5 μm.

U. spisuli spore diameters are 3.0 to 5.9 μm. It is assumed that "diameter" means the shortest axis from outside to outside of spore wall in those cells which are oblate. All species the senior author has examined (*U. crescens*, *U. spisuli*, and *Urosporidium* sp. from trematodes in *Paleomonetes pugio*) are oblate to spherical, not exclusively spherical. All other authors have recorded only "diameters" even though micrographs or drawings of oblate spores are often presented, e.g., Ormières et al. (1973), Caullery and Mesnil (1905).

We consider *U. spisuli* to be a separate species from *U. pelseneeri*, because of host and geographical differences, and the wider range of spore "diameters" in the former. In the absence of a fine structure description of the latter species, further assessments cannot be made. This omission underlines the necessity for ultrastructural studies in elucidating species differences in the genus. A survey of the literature on the genus reveals that in most cases the only substantial reasons for creating a new species have been host and geographical differences. Lengths, and numbers of epispore extensions and spore diameters have shown considerable overlap from species to species. Size of sporocysts and degree of inflation of the basal portion of the epispore extensions are so highly variable as to be of doubtful value. Spore diameter is probably a valid taxonomic character; however, its limitations, as previously recorded, must be recognized. Measurements must be made from living cells rather than from fixed ones since fixation often causes a decrease in size particularly when followed by dehydration. Often it has not been stated whether living cells were measured (Caullery and Chappellier, 1906; Dollfus, 1925; Caullery and Mesnil, 1905; Howell, 1967). The axis measured was not stated for any of the species except the present. In considering all species one finds that spore diameters overlap markedly thus reducing the credibility of spore size as a taxonomic marker (*U. fuliginosum*, Caullery and Mesnil, 1905: 5 μm; *U. pelseneeri*, Caullery and Chappellier, 1906; Dollfus, 1925: 4.5 to 5.5 μm; *U. jiroveci*, Ormières, Sprague, and Bartoli, 1973: 5.5 to 7 μm, $\bar{x} = 6.6$; *U. constantae*, Howell, 1967: 4 to 5 μm; *U. crescens*, DeTurk, 1940: 4.97 to 5.68 μm; *U. tauricum*, Zaika and Dolgikh, 1963: 5.5 to

7.5 μm). It would be useful if all spore sizes were presented giving ranges, means, and standard errors then reasonable judgments could be made.

In the future the greatest potential for elucidation of species differences within the genus will lie in descriptions of fine structure. For example, in the three species examined by electron microscopy it appears that epispore fibrils and ribbons may be useful in distinguishing species. Those of *U. crescens* (Perkins, 1971) and *U. jiroveci* (Ormières et al., 1973) are similar in appearance; however, measurements of periodicities cannot be compared because those of *U. jiroveci* were not published. Those of *U. spisuli* are clearly different from the other two species (compare figs. 12–14, Perkins, 1971, and figs. 16 and 17, Ormières et al., 1973, with Figs. 4 and 5 of the present work). It would be useful to examine the fine structure of all species of *Urosporidium* and in particular the epispore fibers and ribbons. Possibly stronger taxonomic distinctions will emerge from comparisons of this type.

The economic concern caused by sporulation of *U. spisuli* is the second example where sporulation of a hyperparasite has caused problems. As described by Perkins (1971), *U. crescens* infects the metacercaria of the trematode *Carneophallus* sp. which, in turn, infects the blue crab, *Callinectes sapidus*. When sporulation of *U. crescens* occurs, encysted, 0.3 to 1 mm diameter, metacercariae become dark brown or black causing the syndrome known as pepper crab disease which has resulted in crabs being withheld from the market as in the case of *S. solidissima*. In some regions the incidence of "pepper" crabs can be significant as evidenced by an unpublished 1967 to 1968 survey by Van Engle of the Virginia Institute of Marine Science where 53% of crabs around Wachapreague, Virginia, were found with black encysted metacercariae.

At present the life cycle of the nematode and *U. spisuli* are unknown; therefore, predictions as to when or where hyperparasitized worms will appear in clam meats cannot be offered. Even if the life cycle of the surf clam worm were known, the life cycle of *U. spisuli* would probably be in question, because it is unlikely that infections are transmitted from

worm to worm. With the possible exception of Barrow's (1965) studies, no haplosporidan spores have been shown to infect individuals of the same species from which they were obtained (Pixell-Goodrich, 1915; Taylor, 1966). We have not been successful in attempts to infect oysters (*Crassostrea virginica*) with spores of *Minchinia nelsoni* and *M. costalis*, trematode metacercariae (*Carneophallus* sp.) with spores of *Urosporidium crescens*, and *Panopeus herbstii* with spores of *Minchinia* sp.

There appears to be no potential health hazard from ingesting commercially prepared meats of *S. solidissima* which contain *U. spisuli*, because all clams are cooked prior to eating and none are used in the half-shell or raw trade. Processing methods include deep fat frying or retorting clam meats in which temperatures range from approximately 132 C for 8 min to 116 C for 90 min. All of the spores of *U. spisuli* appear to be killed after 5 min at 100 C as determined by the eosin Y dye exclusion test for cell viability (0.17% eosin Y in molluscan physiological saline equivalent to 35‰ seawater). Evans' blue staining (0.25% in estuarine water) (Crippen and Perrier, 1974) indicates that 30 min at 100 C is required before all are killed. Parasitized nematodes removed from host tissues were unable to survive more than 24 hr in molluscan physiological saline at 37 C and were killed in vivo in a commercial processing step employing 132 C for 8 min. Even if the clams were eaten raw it is unlikely that there would be a health hazard from the protozoan. No Haplosporea resembling *Urosporidium* have been reported from vertebrates.

ACKNOWLEDGMENTS

LITERATURE CITED

ANONYMOUS. 1974. Fisheries of the United States, 1973. Statistics and Market News Division, U. S. Dept. Commerce, NOAA, National Marine Fisheries Service. Current Fishery Statistics No. 6400.

BARROW, J. H., JR. 1965. Observations on *Minchinia pickfordae* (Barrow 1961) found in snails of the Great Lakes region. Trans Am Microsc Soc **84**: 587–593.

CAULLERY, M., AND A. CHAPPELLIER. 1906. *Anurosporidium pelseneeri*, n.g. n. sp., haplosporidie infectant les sporocystes d'un trematode parasite de *Donax trunculus* L. C R Soc Biol, Paris **60**: 325–328.

———, AND F. MESNIL. 1905. Sur quelques nouvelles haplosporidies d'annélide. C R Soc Biol, Paris **58**: 580–583.

CRIPPEN, R. W., AND J. L. PERRIER. 1974. The use of neutral red and Evans blue for live-dead determinations of marine plankton. Stain Technol **49**: 97–104.

DETURK, W. E. 1940. The occurrence and development of a hyperparasite, *Urosporidium crescens* sp. nov. (Sporozoa, Haplosporidia) which infests the metacercariae of *Spelotrema nicolli*, parasitic in *Callinectes sapidus*. J Elisha Mitchell Sci Soc **56**: 231–232.

DOLLFUS, R. PH. 1925. Liste critique des cercaires marines à queue sétigère signalées jusqu'à présent. Trav Sta Zool Wimereux **9**: 43–65.

HOWELL, M. 1967. The trematode, *Bucephalus longicornutus* (Manter, 1954), in the New Zealand mud-oyster, *Ostrea lutaria*. Trans R Soc N Z Zool **8**: 221–237.

ORMIÈRES, R., V. SPRAGUE, AND P. BARTOLI. 1973. Light and electron microscope study of a new species of *Urosporidium* (Haplosporida), hyperparasite of trematode sporocysts in the clam *Abra ovata*. J Invertebr Pathol **21**: 71–86.

PERKINS, F. O. 1968. Fine structure of the oyster pathogen *Minchinia nelsoni* (Haplosporida, Haplosporidiidae). J Invertebr Pathol **10**: 287–305.

———. 1969. Electron microscope studies of sporulation in the oyster pathogen, *Minchinia costalis* (Sporozoa: Haplosporida). J Parasitol **55**: 897–920.

———. 1971. Sporulation in the trematode hyperparasite *Urosporidium crescens* DeTurk, 1940 (Haplosporida: Haplosporidiidae)—an electron miscroscope study. J Parasitol **57**: 9–23.

———. 1975. Fine structure of *Minchinia* sp. (Haplosporida) sporulation in the mud crab *Panopeus herbstii*. *In* G. Pauley (ed.), Diseases of Crustaceans. Marine Fish. Rev. U. S. Dept. Commerce, National Marine Fisheries Service **37**: 46–60.

PIXELL-GOODRICH, H. L. M. 1915. *Minchinia*: a haplosporidian. Proc Zool Soc Lond **1915**: 445–457.

SPRAGUE, V. 1970. Some protozoan parasites and hyperparasites in marine bivalve molluscs. P. 511–526, *in* S. F. Snieszko, (ed.), A Symposium on Diseases of Fishes and Shell-

fishes. Spec Publ 5, Am Fish Soc, Washington, D. C.

TAYLOR, R. L. 1966. *Haplosporidium tumefacientis* sp. n., the etiologic agent of a disease of the California sea mussel, *Mytilus californianus* Conrad. J Invertebr Pathol **8**: 109–121.

ZAIKA, V. E., AND A. V. DOLGIKH. 1963. A rare case of hyperparasitism of Haplosporidium of *Urosporidium tauricum* sp. n. in partenites of trematodes of the family Hemiuridae Lühe from the mollusc *Rissoa splendida* Eichw. (In Russian, English summary.) Zool Zh **42**: 1727–1729.

BOOK REVIEW . . .

Animal Agents and Vectors of Disease, by E. C. Faust, P. C. Beaver, and R. C. Jung. 4th Ed. Lea and Febiger, Philadelphia. 1975. ix + 479 p. 201 text figures; 9 plates, 7 in color. $15.00.

Beginning with the first edition in 1955, this text has been an excellent source of basic information on medical parasitology; this edition continues that tradition, with well-written chapters containing high-quality drawings and photographs, and many key references. The text has five sections: General Principles and Orientation (13 pages), Protozoan Agents of Human Disease (107 pages), Helminths as Agents of Human Disease (207 pages), Arthropods as Agents and Vectors (71 pages), and Technical Aids (41 pages).

The chapters on the protozoa in this edition, as compared to the third, include the recognition of *Entamoeba hartmanni* as a distinct species, which is nonpathogenic; new and updated sections on primary amebic meningoencephalitis, *Isospora*, *Toxoplasma*, *Sarcocystis*, *Babesia*, and *Pneumocystis*; and several new photographs of organisms and lesions.

The chapters on the helminths have been updated in terms of new information and references on epidemiology, pathogenesis, and treatment, and several new photographs of organisms and lesions have been added to this edition. The information on the arthropods and technical aids is virtually that of the third edition.

A general comment is that the recent findings on the immunological and pathophysiological aspects of parasitic infections received comparatively little emphasis, e.g., functional changes in the intestine associated with the enterics, immunodiagnosis of congenital toxoplasmosis. Specific comments to be considered by the authors in future editions follow. The term "cuticula" is used to identify the outer surface of flukes and cestodes as a secretion of the hypodermis; "integument" is the preferred term by several workers in view of ultrastructure studies that show the outer surface is distal cytoplasm of cells whose nucleated portion is located among parenchymal cells. The description of calcareous corpuscles leaves one with the impression that these are composed solely of calcium carbonate. Transmammary transmission of *Toxocara canis* and *T. cati* is omitted in comments on routes of infection in dogs and cats, respectively. The distribution stated for *Loxosceles reclusus*, i.e., Missouri and midwestern states, is more restricted than that reported by Butz (1971, Clin Toxicol **4**: 515), who reports it as a major resident in the south central U. S., east from Texas to South Carolina, and south from Indiana to Alabama—excluding Florida. The recently recognized and important role of mites in "house dust" allergy is not included; nor is adequate space given to the Hymenoptera in terms of allergic reactions. Rickettsiae are now generally considered as bacteria and hence should be included in that section. The micrometer has largely replaced the micron as a unit of length in most journals.

This edition will be a useful and comprehensive source of basic information on medical parasitology for medical students, and professionals in several of the biomedical disciplines.

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