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Acoel flatworms (Platyhelminthes) from the ancient Punic Ports of Carthage, at Salammbô, Tunisia

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Abstract: Three new species of acoel flatworms found in the Punic Ports at Salammbô, Tunisia are described. These are *Amphiscolops mosaicus* (Convolutidae), *Symsagittifera poenicea* (Sagittiferidae), and *Paramecynostomum carchedonium* (Mecynostomidae). *Otocelis rubropunctata* (Otocelididae), abundant in the Punic Ports, is also characterized.

Résumé : *Plathelminthes acoeles des anciens ports puniques de Carthage, à Salammbô, Tunisie*
Trois nouvelles espèces d'acoeles des Ports Puniques de Salammbô, Tunisie sont décrites. Ce sont *Amphiscolops mosaicus* (Convolutidae), *Symsagittifera poenicea* (Sagittiferidae), et *Paramecynostomum carchedonium* (Mecynostomidae). *Otocelis rubropunctata* (Otocelididae), abondante dans les Ports Puniques, a été également identifiée.

Keywords : Platyhelminthes, Acoela, Tunisia, Mediterranean, taxonomy.

Introduction

Many species of acoel platyhelminths have been reported from the European side of the Mediterranean Sea. Some are essentially classical species that have been studied carefully by at least one zoologist and that may have been recorded from several localities, even outside the confines of the Mediterranean; others are poorly known with respect to their distribution or morphology.

The fauna of marine platyhelminths of the African side of the Mediterranean has scarcely been explored, and little has been published concerning acoels of this region. Crezée (1975), in a detailed study of the family Solenofilomorphidae, described, but did not name, a species of *Endocincta* from Raoud Beach, Tunisia; he also characterized an unnamed genus and species from Khereddine. Subsequently, Crezée (1978) reported the presence of *Paratomella rubra* Rieger & Ott, a member of the family Paratomellidae, at both Khereddine and Raoud Beach. The type locality of *P. rubra* is Venice, Italy, but this species is known to be widely distributed in the Atlantic, having been found as far west as North Carolina (Tyler, 1973), and it has also been reported from the North Sea. We may expect that as the fauna of acoels on the African coast of the Mediterranean becomes better known, many of the species will be those already reported from the European side and perhaps from more distant regions.

During late January and early February 1971, I had the pleasure of working for three weeks at Institut National Scientifique et Technique d'Océanographie et de la Pêche at Salammbô, Tunisia. During my visit, I made collections in several intertidal and shallow-water situations that were close to Salammbô and that I expected to be populated by acoels. Various platyhelminths were often present in my

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samples, but the only sites where I regularly found acoels were the ancient Punic Ports of Carthage. On subsequent short visits I had additional opportunities to collect and study the acoels occurring in the Punic Ports, and attempted to cultivate certain of them. Altogether, I found five species. One was *Otocelis rubropunctata* (O. Schmidt), whose type locality is in the Adriatic Sea and whose known distribution extends from the Black Sea to the North Atlantic. Two others, belonging to the genera *Amphiscolops* and *Paramecynostomum*, were new and will be described in this paper. A species of *Symsagittifera* will also be described, although it may only be a variant of *S. psammophila* Beklemishev, 1957. Another acoel was so rarely encountered that I was unable to study it properly. I had hoped, in fact, to examine the *Amphiscolops* and *Symsagittifera* again, because my material of these was extremely limited.

Through the centuries, the two lagoons that once served as the military port and mercantile port of Carthage have undergone many changes. For many years they were separated from the sea and from one another, and their salinity varied extensively. In summer, they became hypersaline, and their fauna included the brine shrimp, *Artemia salina*. Today the lagoons are connected by a canal, and each has an opening to the Mediterranean; thus there is an exchange of water with the sea, and the level fluctuates slightly in accordance with tides and weather conditions. The salinity of the water collected at various points in the lagoons in mid-January ranged from 35.9‰ to 36.9‰; the salinity at the shore of the sea was 36.9‰.

The more conspicuous algae in the lagoons are *Ulva lactuca* and *Enteromorpha intestinalis*, both of which form extensive growths near the water's edge, sometimes exposed when the water level drops. The sediment washed from these algae, and also the sediment taken from the muddy sand or clay beneath them, has a rich fauna of small invertebrates, including amphipods, small gastropods (prosobranchs, opisthobranchs), harpacticoid copepods, platyhelminths (acoels, neorhabdocoels, protriclads, triclads, at least one polyclad), and a kinorhynch. Where the mercantile port opens into the sea, there is an area characterized by rather clean sand, although this is usually covered to a large extent by coarse detritus consisting mostly of decomposing *Posidonia oceanica* that has been washed in. This habitat also supports a variety of small invertebrates, including platyhelminths, but in my experience its fauna is less rich than that associated with growths of *Ulva* and *Enteromorpha*.

Methods

Three of the four acoels with which this report is concerned were found in sediment skimmed from the substratum or washed from *Ulva* and *Enteromorpha*; one was found in relatively clean sand between masses of decaying *Posidonia* and other detritus. A solution of magnesium chloride isotonic with sea water was used to quiet specimens that were to be studied alive or fixed in Bouin's solution in preparation for being embedded in paraffin. Transverse, sagittal, and frontal sections were cut at 8 μm and stained with iron hematoxylin.

Results

Family **Convolutidae**

Amphiscolops mosaicus sp. nov.

Figs 1-2

Largest individuals, believed to be fully mature, 4 mm long and 1.1 mm wide when extended and actively gliding in a glass dish; thickness about half of width. Anterior end rounded, posterior end bilobed (Figs 1A, B, E, G). Shape changeable; movements often somewhat peristaltic, but ventral surface tending to remain flat except when worm swims, in which case body becomes more slender and more nearly cylindrical.

Color in strong reflected light olive-green, due to presence of zooxanthellae. Concentrations of iridescent crystals in vacuoles of dorsal epidermis (Figs 1C, 2A, B, D) forming almost continuous, mirrorlike network (Fig. 1B). Combination of zooxanthellae and crystals making body nearly opaque. Zooxanthellae (Figs 1A, 2A-D) rather evenly distributed, but fewer in posterior one-fifth, where limited to region dorsal to vacuolated tissue characteristic of this portion of body, and absent from narrow hyaline zone extending around margin of worm. Distinctly separate zooxanthellae (Fig. 1C) spherical, mostly 15-20 μm in diameter; those in clusters usually somewhat irregular in shape.

Statocyst (Fig. 1E, F) located about 350 μm from anterior end of body; cavity enclosing statolith and smaller cavities flanking it appearing to be enclosed by a common membrane. Eyespots (Figs 1E, 2A), at approximately same transverse level as statocyst, consisting of crowded brick-red granules. Frontal gland not observed.

Epidermal cilia about 8 μm long. Stiff sensory cilia (Fig. 1D), 16 to 18 μm long, scattered along margin of body. Aggregates of rhabdoids (Fig. 1D) conspicuous in epidermis; individual rhabdoids needlelike, 12-13 μm long. Lipid inclusions neither abundant nor conspicuous, but numerous greenish, refractile bodies about 1.3 to 2.6 μm in diameter present beneath epidermis.

Mouth (Fig. 1E) near middle of ventral surface. Digestive syncytium containing harpacticoid copepods and other small crustaceans or crustacean larvae, therefore reddish or brownish because of carotenoid pigments.

Reproductive system incompletely analysed (only two mature specimens available for brief study, these not sectioned). Male pore, covered by small triangular flap of tissue originating on its posterior side (Fig. 1G), located within shallow ventral depression, the posterior edge of this depression about 150 μm from cleft between caudal lobes. Mass anterior to genital pore, approached by sperm channels, believed to be seminal vesicle (Fig. 1G), but its structure not studied. Dense aggregation of granules within seminal vesicle believed to be associated with copulatory organ, but this structure not discerned. Sperm (Fig. 1I), escaping through ruptures in body of compressed specimens, about 300 μm long, very slender and undulating actively throughout posterior four-fifths of length.

Female genital pore not observed. Seminal receptacle (Fig. 1E, G) transversely oval, 200 μm by 100 μm, with about 10 nozzles, some shorter than others (or perhaps broken when specimens compressed). Longer nozzles about 150 μm long, of nearly uniform width, but with perceptible annulations and irregularities (Fig. 1H). Oocytes (Fig. 1E) in two masses, one on each side of midline.

Holotype. United States National Museum (USNM) no. 178818; serial sagittal sections of an immature specimen collected in the military port at Salammbô, Tunisia. This specimen does not show any of the reproductive structures observed in the two nearly mature living individuals on which the description is based.

Etymology. The species name, based on late Latin *mosaicus*, alludes to the combination of zooxanthellae and iridescent crystals, which create a striking pattern reminiscent of ancient mosaics.

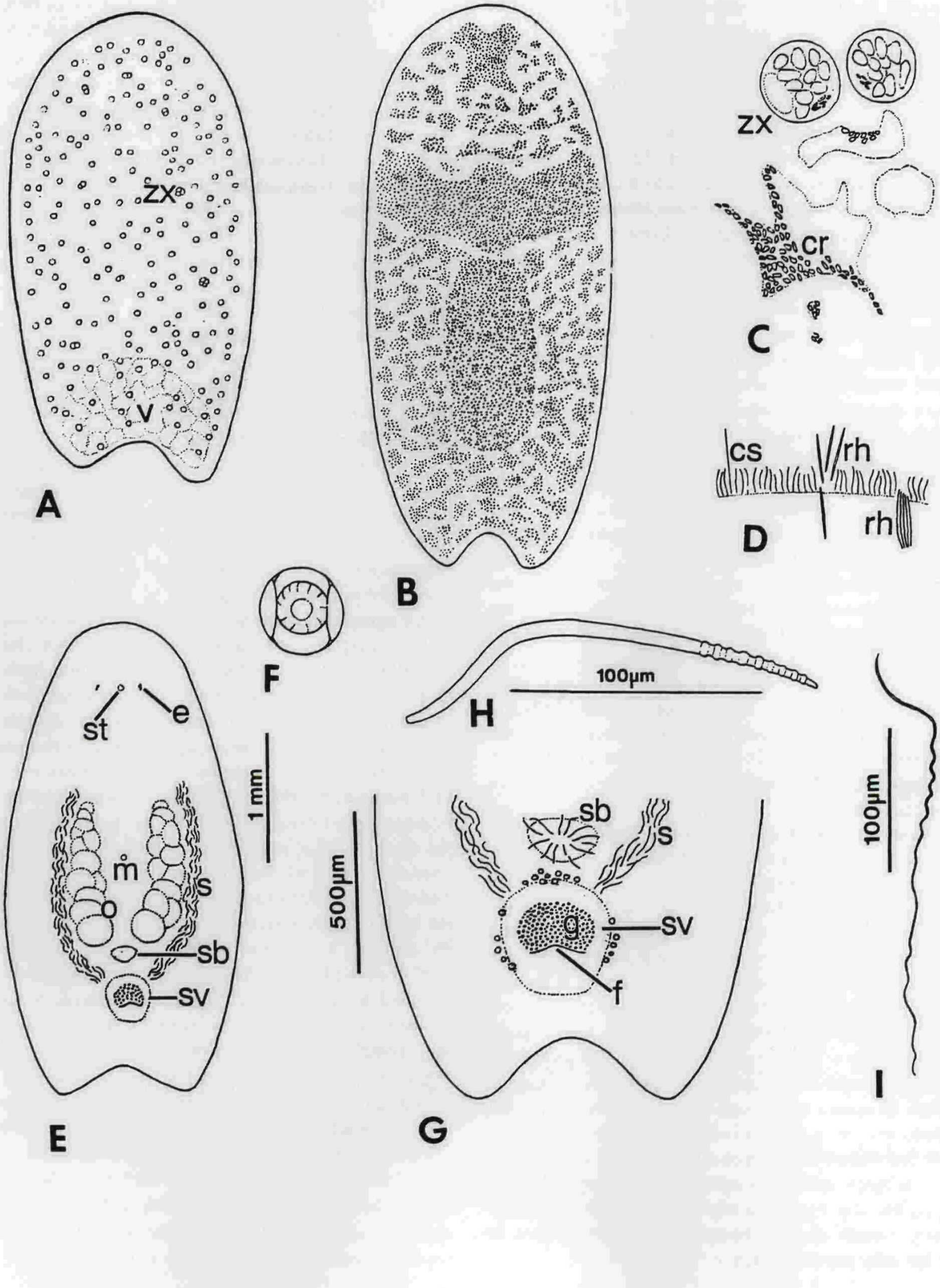
Remarks. *Amphiscolops mosaicus* was found in the sediment accumulating on thalli of *Ulva*, and also in the sediment covering sand and clay where Ulva grows. I collected it in both the mercantile port and military port. Because the two nearly mature specimens could be studied for only a limited time after being immobilized and compressed, my description is somewhat tentative. Nevertheless, this species, even when immature, is so distinctive that it will not be confused with any other known species of *Amphiscolops*.

Specimens were kept alive for several months by feeding them harpacticoid copepods. They multiplied to some extent, but all of the progeny were produced asexually. In cultures, as well as in nature, worms with a bud on the dorsal surface were commonly encountered. After a bud had developed to the point of being a juvenile worm, it became detached, and in the presence of sufficient food it usually grew to a length of about 3 mm, the usual size of the largest immature specimens collected in the field. It seems likely that development of the reproductive system of *A. mosaicus*, and also of several other species of *Amphiscolops* known only from immature specimens, depends on environmental conditions that are not often met. Because the two largest specimens I studied were collected in October, and because none of the many individuals collected in January, February, or March had any trace of a reproductive system, summer and early autumn would probably be the best seasons to search for fully mature worms.

Winsor (1990) published a useful synopsis of named species of *Amphiscolops*, and also included some that have not yet been formally described. Six of the named species (five listed by Winsor, plus *A. marinelliensis* Beltagi & Khafagi, 1984) are characterized by a bilobed posterior end. The one to which *A. mosaicus* seems most closely related is *A. langerhansi* (Graff), known to occur on the European side of the Mediterranean and also at Tenerife (Canary Islands) and Madeira. Hanson (1967) recorded its presence on seagrasses in coves near the Bermuda Biological Station, and the worm was established, at least for a time, in aquaria at the Bermuda Biological Station (Hyman, 1937; Hanson, 1960) and in holding tanks at the School of Marine and Atmospheric Sciences at Miami (Taylor, 1971). On the basis of descriptions and figures of *A. langerhansi* published by Graff (1904, 1904-08) and Hyman (1937), this worm appears to be more slender than *A. mosaicus*. Its patches of crystalline material are mostly large, discrete, and symmetrically arranged, whereas in *A. mosaicus* they are relatively small and form a nearly continuous networklike complex. Furthermore, descriptions of *A. langerhansi* do not mention the mirrorlike character of the patches. The nozzles on the seminal bursa of *A. langerhansi* have prominently enlarged bases, whereas the nozzles of *A. mosaicus* are of nearly uniform width throughout. The tripartite structure of the statocyst of *A. mosaicus* is another distinctive feature of this species.

All immature specimens of *A. mosaicus* that I studied had neither eyespots nor a statocyst, and I could not identify a frontal gland in live worms or in serial sections. In the light of the work of Hanson (1960) on transverse fission by immature specimens of *A. langerhansi*, the absence of a statocyst in species of *Amphiscolops* appears not to be an especially important taxonomic character. Because worms 2 to 3 mm long in Hanson's cultures lacked a statocyst, he proposed that asexual reproduction by fission could explain the absence of this structure in at least some specimens. He cited the work of Keil (1929), who found that when *Polychaerus caudatus*, another large convolutid acoel, was cut in two transversely, the posterior portion regenerated but did not form a statocyst. In his subsequent paper, Hanson (1967) showed that if the statocyst of *A. langerhansi* is removed surgically, it will not be regenerated. A statocyst can be transplanted from one worm to another, however, so long as the implant is positioned in tissue peripheral to the digestive syncytium. Eyepsots regenerate readily.

Hanson (1960) did not observe division of *A. langerhansi* during daylight hours. He therefore reasoned that it took place in darkness, and he in fact saw what appeared to be the start of the division process in two individuals that had been in the dark. The type of budding I have described in *A. mosaicus* is not inhibited by light, and I have never seen any evidence of transverse fission in this species.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The only other Mediterranean *Amphiscolops* that have a bilobed posterior end are two almost certainly separate species whose regeneration was studied by Steinböck (1954, 1955). These worms, collected at three localities on the Spanish coast, were not sexually mature, so Steinböck did not attempt to describe them or assign them to previously named species. He gave the name *forma castellonensis* to the ones from El Grao de Castellón and Palma de Mallorca; they were up to a little more than 3 mm long, yellowish brown, and had symbiotic zooxanthellae and brownish eyespots. The worms from Blanes, up to 4 mm long, green, with zoochlorellae and gray eyespots, were called *forma gerundensis*. Neither type had a statocyst. It is not likely that either of the two kinds of worms found by Steinböck was the same as the one in the Punic ports, which is olive green and characterized by mirrorlike concentrations of crystals.

Four of five undescribed species of *Amphiscolops* found by Yamasu and Okazaki (1987) in the Ryukyu Islands have a bilobed posterior end, but none is similar to *A. mosaicus*. An unnamed *Amphiscolops* with a posterior cleft was reported by Ehlers and Dörjes (1979) from Santa Cruz, Galápagos Islands. The immature specimens available to them were similar to immature *A. mosaicus* in general shape and in lacking a statocyst, but had a pair of eyespots. The coloration imparted by the zooxanthellae was gold-brown, and the anteriormost part of the body had some dark pigment. No mention was made of crystalline inclusions. An *Amphiscolops* found in aquaria at Georgetown University (Blanco & Chapman, 1987) was said to resemble *A. langerhansi*, but thought to be a new species. Apparently no further studies on this acoel have been published.

Family **Sagittiferidae**

Symsagittifera poenicea sp. nov.

Figs 3-4

Largest individuals (Fig. 3A), when extended and gliding, 940 μm long, 310 μm wide; thickness, near end of first one-third of body length (Fig. 3C), about two-thirds of width, decreasing anteriorly and posteriorly. Worms very active, constantly lifting anterior end and also exhibiting twitching movements; usually remaining in tight contact with substratum, but occasionally swimming free.

Anterior end rounded, posterior end rounded or nearly truncate, the variation in shape related in part to disposition of lengthwise flaps of tissue bordering ventral groove (Fig. 3B).

Figure 1. *Amphiscolops mosaicus* sp. nov.; drawings based on sketches of living specimens (E, G, H, I prepared with the aid of a camera lucida).

(A) Immature specimen approximately 1 mm long, dorsal view, showing general shape of body, vacuolated posterior region (*v*), and distribution of zooxanthellae (*zx*).

(B) Immature specimen approximately 2.5 mm long, dorsal view, showing general shape of body and distribution of crystalline inclusions in dorsal epidermis.

(C) Crystalline inclusions (*cr*), in vacuoles of dorsal epidermis, and subepidermal zooxanthellae (*zx*).

(D) Sensory cilium (*cs*), motile cilia, and rhabdoids (*rh*) at margin of body.

(E) Mature specimen approximately 4 mm long, ventral view, showing statocyst (*st*), eyespots (*e*), mouth (*m*), oocytes (*o*), seminal bursa (*sb*), sperm channels (*s*), and seminal vesicle (*sv*).

(F) Statocyst of mature specimen. The cavity enclosing the statolith is flanked by two smaller cavities. All three cavities are enclosed by a common membrane.

(G) Posterior portion of mature specimen, ventral view, showing flap (*f*) covering male genital pore, channels of sperm (*s*) approaching seminal vesicle (*sv*), secretory granules (*g*) in cells believed to be associated with male copulatory organ, and seminal bursa (*sb*) with nozzles.

(H) Nozzle of seminal bursa. Annulations shown only in distal portion.

(I) Living sperm, in fresh smear.

Figure 1. *Amphiscolops mosaicus* sp. nov.; dessins basés sur esquisses de spécimens vivants ; E, G, H, I dessinés à la chambre claire.

(A) Spécimen juvénile, 1 mm de longueur environ, vue dorsale montrant la forme générale du corps, la région postérieure vacuolisée (*v*), et la distribution des zooxanthelles (*zx*).

(B) Spécimen juvénile, 2,5 mm de longueur environ, vue dorsale montrant la forme générale du corps et la distribution des inclusions cristallines de l'épiderme dorsal.

(C) Inclusions cristallines (*cr*) dans les vacuoles de l'épiderme dorsal, et zooxanthelles (*zx*) sous-épidermiques.

(D) Cil sensoriel (*cs*), ciliature épidermique, et rhabdoïdes (*rh*) au bord du corps.

(E) Spécimen mûr, 4 mm de longueur environ, vue ventrale montrant le statocyste (*st*), les taches oculaires (*e*), la bouche (*m*), les ovocytes (*o*), la bourse copulatrice (*sb*), les canaux spermatiques (*s*), et la vésicule séminale (*sv*).

(F) Statocyste d'un spécimen mûr. La cavité qui contient le statocyste est flanquée de deux cavités plus petites ; les trois cavités sont entourées par une membrane commune.

(G) Portion postérieure d'un spécimen mûr, vue ventrale montrant le repli (*f*) qui couvre le pore génital mâle, les canaux spermatiques (*s*) débouchant dans la vésicule séminale (*sv*), les grains de sécrétion (*g*) des cellules qui entourent l'organe mâle copulateur, et la bourse copulatrice (*sb*) avec ses canules.

(H) Une canule de la bourse copulatrice. La surface annelée n'est répresentée que dans la partie distale.

(I) Spermatozoïde vivant, dans un frottis frais.

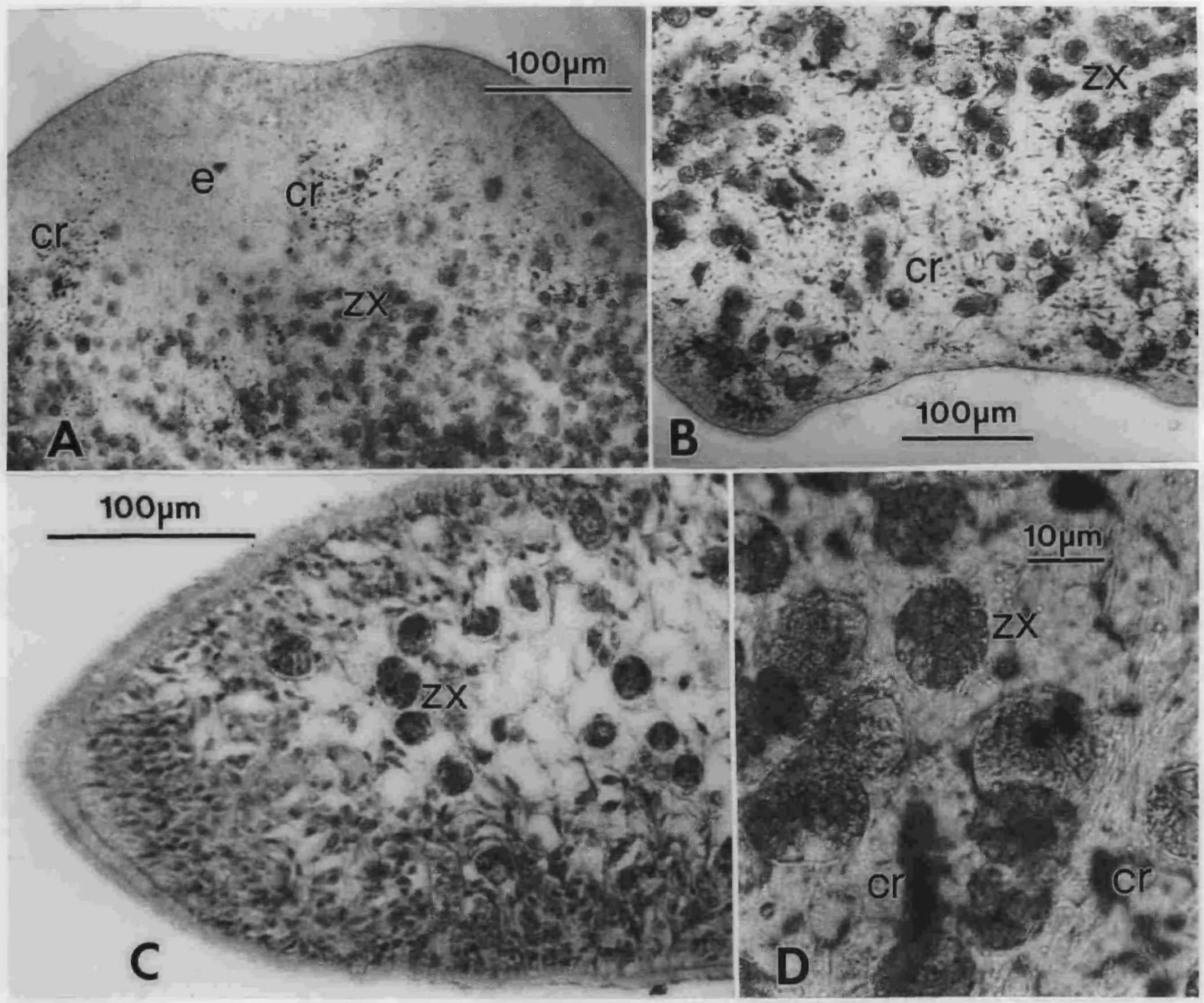

Figure 2. *Amphiscolops mosaicus* sp. nov.; photomicrographs of immature specimens.
(A) Anterior portion of living specimen, dorsal view, showing eyespots (*e*), zooxanthellae (*zx*), and crystals (*cr*) in epidermis.
(B) Posterior portion of living specimen, dorsal view, showing zooxanthellae (*zx*) and crystals (*cr*).
(C) Anterior portion, sagittal section, of fixed specimen stained with iron hematoxylin. *zx* zooxanthellae.
(D) Portion from near posterior end of living specimen, showing zooxanthellae (*zx*) and concentrations of epidermal crystals (*cr*).
Figure 2. *Amphiscolops mosaicus* sp. nov.; photomicrographies des spécimens jeunes.
(A) Partie antérieure d'un spécimen vivant, vue dorsale montrant les taches oculaires (*e*), les zooxanthelles (*zx*), et les cristaux épidermiques (*cr*).
(B) Partie postérieure d'un spécimen vivant, vue dorsale montrant les zooxanthelles (*zx*) et les cristaux épidermiques (*cr*).
(C) Partie antérieure d'un spécimen fixé, coupe sagittale, colorée par l'hématoxyline ferrique. (*zx*) zooxanthelles.
(D) Partie près de l'extrémité postérieure d'un spécimen vivant, montrant les zooxanthelles (*zx*) et les concentrations de cristaux épidermiques (*cr*).

Opening between flaps widest in posterior half of body, but flaps nearly touching near middle or slightly anterior to middle; space between them generally widening again anteriorly.

Color dark green in reflected light, owing to zoochlorellae (Figs 1A, D, E, 4A, B) concentrated just beneath epidermis. Zoochlorellae mostly 9-11 μm in diameter, bright green in transmitted light.

Statocyst (Figs 3E, 4A) 18-20 μm in diameter in largest worms. Eyespots not observed, and probably absent. Frontal gland relatively small, not reaching transverse level at which statocyst is located. Motile epidermal cilia about 8 μm long; stiff sensory cilia 12 μm long also present. Aggregates of rhabdoids (Fig. 3D, E), about 20 μm long and pale brick-red, rather evenly distributed over body surface. Male genital pore (Fig. 3D, E) located on ventral surface near beginning of posterior one-sixth of body. Seminal vesicle (Fig. 3E), more obvious in sections than in living specimens, thin-walled, with substantial accumulation of sperm above inverted copulatory organ. Packets of sperm and developing sperm located on both sides of middle one-third of body. Mature sperm (Fig. 3F) about 215 μm long, 4 μm thick slightly posterior to middle; anterior one-sixth to one-third of sperm slightly twisted and lacking conspicuous inclusions; remainder, except for very slender posterior portion, with crowded small granules.

Female genital pore (Fig. 3E), in front of male pore, leading anterodorsally to rather long and muscular vagina, this joined to approximately spherical to ellipsoidal seminal bursa (Fig. 3D, E); nozzle of bursa (Figs 3D, 4B) sclerotized, 60-75 μm long, directed ventrally and to left, then curving forward. Smaller oocytes, obscured by zoochlorellae and difficult to see in living worms, beginning at about same transverse level as anteriormost packets of developing sperm, but ventral to these. At least two large oocytes (Fig. 3A), sometimes four or six, typically present just anterior to seminal bursa in large specimens.

Sagittocysts (Figs 3D, E, 4B), these narrowly spindle-shaped, up to about 35 μm long, and stained intensely by hematoxylin, present close to ventral epidermis at posterior end of body, around male genital pore, just posterior to seminal bursa, and lateral to seminal bursa and seminal vesicle.

Holotype. USNM no. 178819; a nearly complete series of sagittal sections of a worm collected in the mercantile port at Salammbô, Tunisia.

Etymology. The species name is based on Latin *poeniceus*, meaning Punic or Carthaginian.

Remarks. This acoel was found sparingly at the mouth of the mercantile port, in relatively clean sand between masses of decaying *Posidonia* and other deposited material.

Dörjes (1968) recognized 31 species of *Convoluta*, and since his valuable monograph was published, several others were added to the genus. Kostenko & Mamkaev (1990a) proposed that species characterized by the presence of sagittocysts and zoochlorellae (*C. schultzei* O. Schmidt; *C. roscoffensis* Graff; *C. japonica* Kato; *C. macnaei* du Bois-Reymond Marcus; *C. psammophila* Beklemishev; *C. bifoveolata* Mamkaev; *S. nitidae* Yamasu) be placed in a separate genus, *Simsagittifera*. Soon afterward, Kostenko & Mamkaev (1990b) decided that *Simsagittifera*, with the seven species just listed, should be assigned to a new family, Sagittiferidae. In the same paper they also described the genus *Praesagittifera* to accommodate a new species, *P. shikoki*, as well as *Convoluta gracilis* Yamasu and *C. naikaiensis* Yamasu. All three have zoochlorellae, but lack sagittocysts. *Convoluta sagittifera* Ivanov, which has sagittocysts but lacks zoochlorellae, was assigned to the new genus *Sagittifera*. Subsequently, Mamkaev & Kostenko (1991) emended the genus name *Simsagittifera* to *Symsagittifera*.

The many acoels previously assigned to *Convoluta* are sufficiently diverse that it is logical to distribute them in several genera, and even in more than one family. It is also reasonable to invoke the presence or absence of sagittocysts for defining genera and families. Kostenko & Mamkaev 1990 have probably laid too much stress, however, on the presence or absence of zoochlorellae. In various groups of invertebrates, there are morphologically unified genera that include some species with zoochlorellae or zooxanthellae, others without them. *Paramecium* and *Stentor*, among the ciliates, and *Anthopleura*, among the sea anemones, are good examples.

Symsagittifera poenicea differs only slightly from *S. psammophila* Beklemishev (1957). In his original description of *S. psammophila* from Feodosia, on the Ukrainian side of the Black Sea, Beklemishev stated that there were sagittocysts on both sides of the mouth region; these were designated oral sagittocysts and were shown in one of the illustrations. Kostenko and Mamkaev (1990a), who carefully studied specimens from other localities on the Ukrainian side of the Black Sea, also described and illustrated substantial concentrations of sagittocysts lateral to the mouth. None of the specimens of *S. poenicea* that I observed in the living state or in sections had sagittocysts in this region of the body. The drawing of a ventral view of *S. psammophila* published by Kostenko & Mamkaev does not show sagittocysts lateral to the seminal bursa, where they are definitely present in *S. poenicea*. Furthermore, the largest specimens I found at Salammbô (940 μm long) were appreciably smaller than the largest specimens observed by Kostenko & Mamkaev (1.4 mm long); Beklemishev did not give the size of mature specimens he studied at Feodosia.

Together with his description of *C. psammophila*, Beklemishev stated that he found two specimens at Posillipo, near Naples, Italy, in 1927. In these, the two pairs of dorsal longitudinal nerves were clearly visible, whereas these nerves could not be seen in specimens from the Black Sea. The worms from Posillipo also had a prominent frontal gland, and the eyespots and rhabdoids were comparatively more yellowish. Nevertheless, his drawing of an immature specimen shows sagittocysts at the level of the mouth, as is typical of *S. psammophila* in the Black Sea.

An acoel identified as *S. psammophila* has been intensively studied by biologists at Università di Pisa (recent publications in the series are those of Bedini & Lanfranchi, 1991 and Ferrero & Bedini, 1991), but their

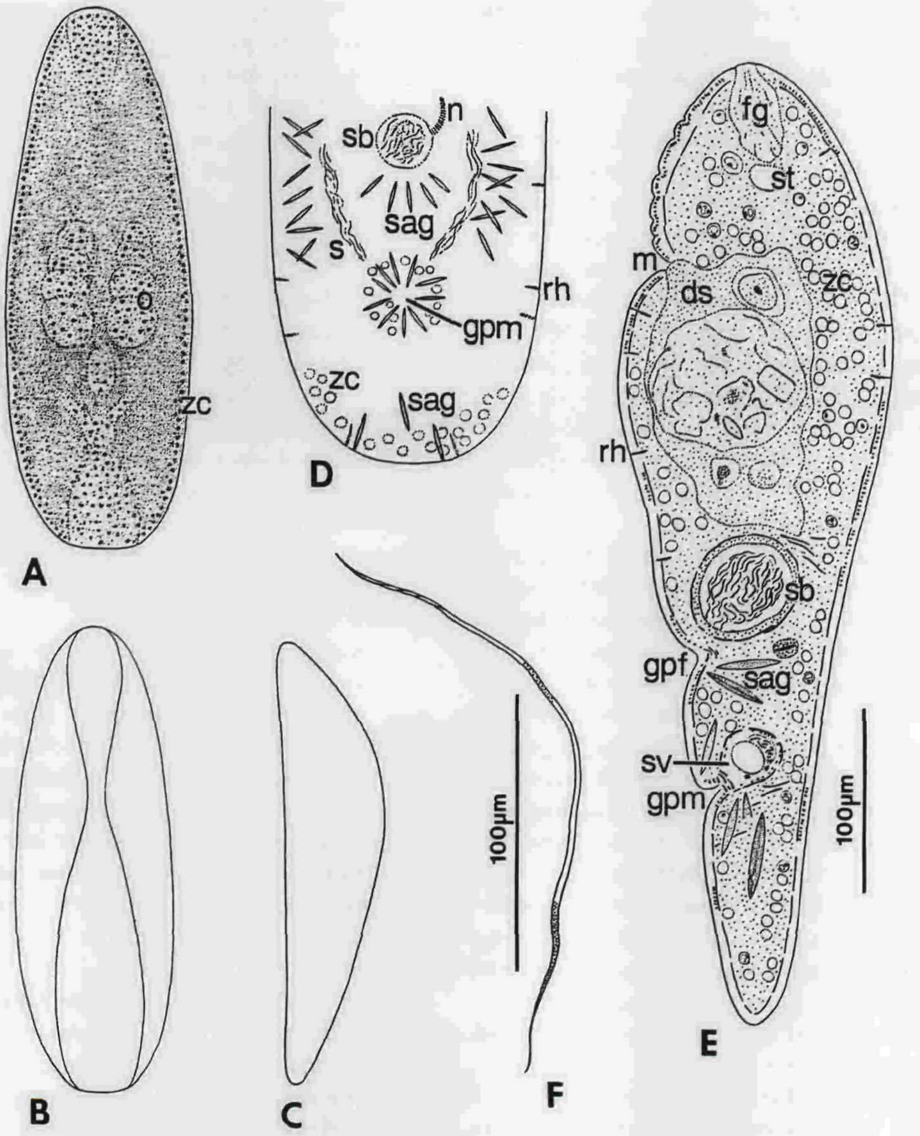

Figure 3. *Symsagittifera poenicea* sp. nov., drawings of living and sectioned specimens.
(A) Living specimen, dorsal view, showing oocytes (*o*) and some zoochlorellae (*zc*).
(B) Living specimen, ventral view, outline drawing showing ventral folds of body.
(C) Living specimen, lateral view from left side, outline drawing showing profile of body.
(D) Living specimen, posterior portion, ventral view, showing seminal bursa (*sb*) and its nozzle (*n*), sperm channels (*s*), male genital pore (*gpm*), rhabdoids (*rh*), sagittocysts (*sag*), and some zoochlorellae (*zc*).
(E) Composite drawing based on several serial sagittal sections stained with iron hematoxylin; prepared with the aid of a camera lucida. (*ds*) digestive syncytium, (*fg*) frontal gland, (*gpf*) female genital pore, (*gpm*) male genital pore, (*m*) mouth; (*rh*) rhabdoids, (*sag*) sagittocysts, (*sb*) seminal bursa, (*st*) statocyst, (*sv*) seminal vesicle, (*zc*) zoochlorellae.
(F) Living sperm, in fresh smear.

Figure 3. *Symsagittifera poenicea* sp. nov., dessins de spécimens vivants et en coupes histologiques.
(A) Spécimen vivant, vue dorsale montrant des ovocytes (*o*) et quelques zoochlorelles (*zc*).
(B) Spécimen vivant, vue ventrale, schéma montrant les replis ventraux.
(C) Spécimen vivant, vue du côté gauche, schéma montrant le profil du corps.
(D) Spécimen vivant, partie postérieure, montrant la bourse copulatrice (*sb*) et sa canule (*n*), les canaux spermatiques (*s*), le pore génital mâle (*gpm*), les rhabdoïdes (*rh*), les sagittocystes (*sag*), et quelques zoochlorelles (*zc*).
(E) Reconstruction d'après des dessins à la chambre claire de coupes sagittales colorées par l'hématoxyline ferrique. (*ds*) syncytium digestif, (*fg*) glande frontale, (*gpf*) pore génital femelle, (*gpm*) pore génital mâle, (*m*) bouche, (*rh*) rhabdoïdes, (*sag*) sagittocystes, (*sb*) bourse copulatrice, (*st*) statocyste, (*sv*) vésicule séminale, (*zc*) zoochlorelles.
(F) Spermatozoïde vivant, dans un frottis frais.

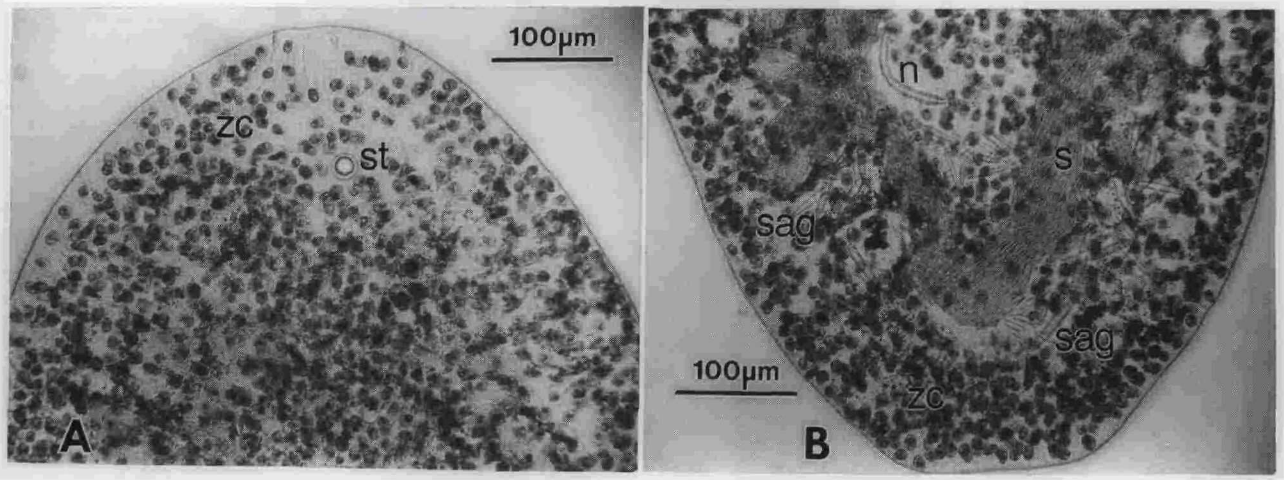

Figure 4. *Symsagittifera poenicea* sp. nov., photomicrographs of living specimen.
(A) Anterior end, dorsal view, showing statocyst (*st*) and distribution of zoochlorellae (*zc*).
(B) Posterior end, ventral view, showing nozzle (*n*) of seminal bursa, sperm channels (*s*), sagittocysts (*sag*), and zoochlorellae (*zc*).
Figure 4. *Symsagittifera poenicea* sp. nov.; photomicrographies d'un spécimen vivant.
(A) Partie antérieure, vue dorsale montrant le statocyste (*st*) et la distribution des zoochlorelles (*zc*).
(B) Partie postérieure, vue ventrale montrant la canule (*n*) de la bourse copulatrice, les canaux spermatiques (*s*), les sagittocystes (*sag*), et les zoochlorelles (*zc*).

papers deal with ultrastructure of the epidermis, nervous system, sensory cilia, statocyst, and zoochlorellae, rather than with characters that would be helpful in taxonomy. If enough specimens of the *S. psammophila*-type from various localities in the Black Sea and Mediterranean were studied, the disposition of sagittocysts would perhaps prove to be so variable as to be of no systematic importance. In that case *S. poenicea* would become a junior synonym of *S. psammophila*.

Family **Mecynostomidae**
Paramecynostomum carchedonium sp. nov.
Figs 5-6

Largest specimens, when extended and actively gliding, about 760 µm long, 175 µm wide near end of first one-third, tapering gradually toward both ends (Figs 5A, 6A); thickness about one-half width.

Living worms, in reflected light, with conspicuous purplish red median streak in anterior one-third or two-fifths of body (Figs 5A, 6A); pigment concentrated in intercellular spaces below dorsal epidermis. Small patches of purplish red pigment also usually evident in posterior one-third of body. Pale yellowish pigment present near margins of area lateral and anterior to statocyst, sometimes continuous and intense enough to form crescent-shaped area. Digestive syncytium (Fig. 5C) mostly pale greenish yellow except where diatoms and other food inclusions (some of these appearing to be amorphous) present.

Statocyst (Figs 5A, 6A), situated near end of first one-tenth of body, about 18 µm in diameter. Frontal gland (Fig. 5C) well developed. Epidermal cilia about 9 µm long. Stiff sensory cilia 10-13 µm long scattered along margins of body. Refractile concretions, or aggregates of small particles with refractile properties, beneath dorsal epidermis. Posterior region of body, lateral to and behind copulatory organs, conspicuously vacuolated (Figs 5A-C, 6A, B). Rhabdoids not observed.

Male genital pore (Fig. 5B, C), on ventral surface near beginning of last one-seventh of body length, leading to bulbous copulatory organ (Figs 5A-C, 6A, B). Gland cells evident among cells arranged centripetally around narrow lumen of copulatory organ. Seminal vesicle absent, but sperm channels (Fig. 5A, B) approaching dorsal side of copulatory organ dilated, thus forming false seminal vesicle. Packets of sperm and developing sperm lateral, beginning not far behind level of statocyst. Sperm (Fig. 5D) about 70 µm long, 1.3 µm thick; tail relatively short, not distinctly demarcated from rest of sperm.

Female genital pore (Fig. 5C), more difficult to see than male pore in living specimens, on ventral surface slightly anterior to male copulatory organ. Vagina (Fig. 5C) somewhat bulbous, leading to seminal bursa (Fig. 5A-C) containing active sperm. Nozzle (Fig. 5C) of bursa, fibrous or fibromuscular and stained intensely by hematoxylin, directed ventrally. Chain of progressively larger oocytes (Figs 5A, B, 6A) on both sides of body. Largest oocytes, close to anterior edge of bursa, about 65 µm in diameter.

Holotype. USNM no. 178820; a series of sagittal sections of a worm collected in the military port at Salammbô, Tunisia.

Etymology. The species name is based on Latin *carchedonius*, meaning Carthaginian.

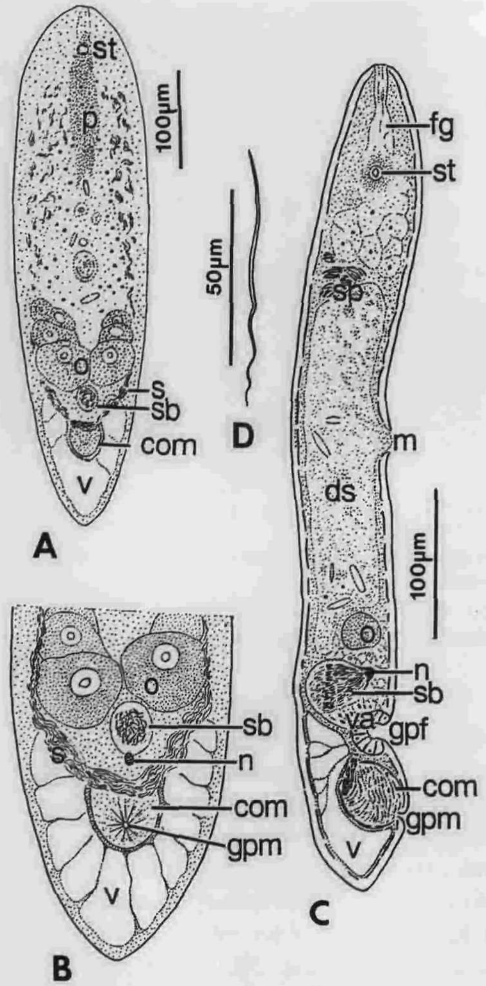

Figure 5. *Paramecynostomum carchedonium* sp. nov.

(A) Entire living specimen, dorsal view, showing statocyst (*st*), streak of purplish red pigment (*p*), oocytes (*o*), seminal bursa (*sb*), sperm channels (*s*), male copulatory organ (*com*), and vacuolated area (*v*) at posterior end.

(B) Posterior portion of living specimen (slightly flattened), ventral view, showing oocytes (*o*), seminal bursa (*sb*) and its nozzle (*n*), male genital pore (*gpm*) and copulatory organ (*com*), dilated sperm channels (*s*) forming false seminal vesicle, and vacuolated area (*v*).

(C) Drawing based on several successive slightly oblique sagittal sections stained with iron hematoxylin; prepared with the aid of a camera lucida. (*ds*) digestive syncytium, (*fg*) frontal gland, *gpf* female genital pore, (*m*) mouth, (*sp*) developing sperm, (*st*) statocyst, (*va*) vagina. Other letters as in B.

(D) Living sperm, in fresh smear.

Figure 5. *Paramecynostomum carchedonium* sp. nov.

(A) Spécimen vivant, vue dorsale montrant le statocyste (*st*), la tache pigmentaire rouge-pourpre (*p*), les ovocytes (*o*), la bourse copulatrice (*sb*), les canaux spermatiques (*s*), l'organe copulateur mâle (*com*), et la région postérieure vacuolisée (*v*).

(B) Partie postérieure d'un spécimen vivant (légèrement comprimé), vue ventrale, montrant les ovocytes (*o*), la bourse copulatrice (*sb*) et sa canule (*n*), le pore génital mâle (*gpm*), l'organe copulateur mâle (*com*), les canaux spermatiques (*s*) dilatés, formant une fausse vésicule séminale, et la région vacuolisée (*v*).

(C) Reconstruction d'après des dessins à la chambre claire de coupes sagittales consécutives, légèrement obliques, colorées par l'hématoxyline ferrique. (*ds*) syncytium digestif, (*fg*) glande frontale, (*gpf*) pore génital femelle, (*m*) bouche, (*sp*) spermatozoïdes en voie de développement, *st* statocyste, *va* vagin. Les autres abréviations comme en B.

(D) Spermatozoïde vivant, dans un frottis frais.

Remarks. *Paramecynostomum carchedonium* was common in sediment washed from *Ulva* and *Enteromorpha* collected at several stations within the Punic Ports. It is an active worm, with steady movements, and it tends to cling tightly to the substratum.

This acoel is probably closely related to *P. diversicolor* (Ørsted). Originally described as *Aphanostoma diversicolor*, it was transferred by Westblad (1946) to *Paraconvoluta*, and then returned by Westblad (1948) to *Aphanostoma*. Dörjes (1968) concluded that it could not be assigned to *Aphanostoma*, whose type species, *A. virescens* Ørsted, belongs to the family Convolutidae. He created for it the genus *Paramecynostomum*, and placed this, along with some other genera, in a new family, Mecynostomidae.

Paramecynostomum diversicolor has been found in the Black Sea, Mediterranean, and Adriatic, as well as in Norway, Sweden, Iceland, Irish Sea, English Channel, and localities along the Atlantic coast of the United States. Reaching a length of about 1.2 mm, it is larger than *P. carchedonium*, and its coloration is more striking. The anterior region is usually conspicuously yellow or orange, and there is a streak of violet pigment, concentrated in cells beneath the epidermis, extending from a level just in front of the statocyst nearly to the middle of the body. A streak, small mass, or V-shaped configuration of violet pigment is also often present near the posterior end. The shape of *P. diversicolor*, more slender in the anterior half of the body than in the posterior half, is also different from that of *P. carchedonium*. On the basis of figures of the reproductive system published by Graff (1891, 1904-08) and Westblad (1946), I judge that the male copulatory complex of *P. diversicolor* has a rather distinct seminal vesicle. In *P. carchedonium*, there is scarcely any lumen in a comparable position; instead, sperm accumulate in dilated portions of the sperm channels, just before these enter the copulatory organ.

Family **Otocelididae**

Otocelis rubropunctata (O. Schmidt)

Figs 7-8

Largest living specimens (at Salammbô), when active and extended, 620 μm long, 285 μm wide. Outline of body (Figs 7A, 8), as seen from dorsal or ventral side, narrowly oval; anterior end broadly rounded, but usually with slight indentation where pore of frontal gland is located; posterior end nearly pointed. Coloration, in life, mostly translucent whitish, but area occupied by digestive syncytium pale olive. Worms capable of adhering tightly to glass, but more often swimming freely or gliding on surface film.

Statocyst (Figs 7A, C, 8), located near the end of first one-eighth of the body, 19 μm in diameter. Eyespots (Figs 7A, 8), consisting of granules of purplish red pigment, on lateral margin of both sides of body slightly anterior to level of statocyst. Each eyespot about 25 μm long, becoming wider posteriorly.

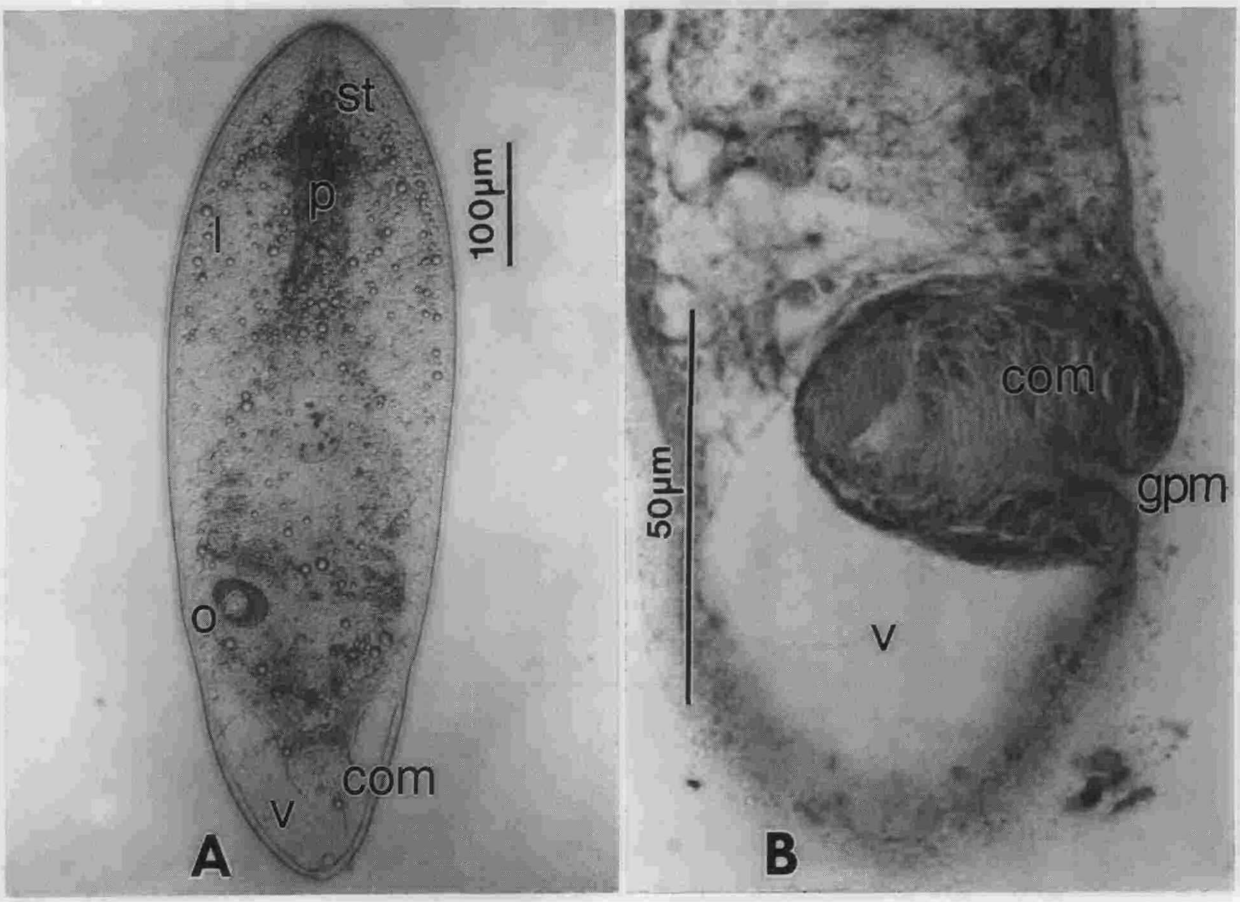

Figure 6. *Paramecynostomum carchedonium* sp. nov., photomicrographs of living specimens.

(A) Entire living specimen, dorsal view, showing statocyst (*st*), streak of purplish red pigment (*p*), lipids (*l*), oocytes (*o*), male copulatory organ (*com*), and vacuolated region (*v*) at posterior end.

(B) Posterior portion of body, sagittal section, stained with iron hematoxylin, showing male genital pore (*gpm*), copulatory organ (*com*), and vacuolated region (*v*) at posterior end.

Figure 6. *Pseudomecynostomum carchedonium* sp. nov.

(A) Spécimen vivant, vue dorsale montrant le statocyste (*st*), la tache de pigment rouge-pourpre (*p*), les globules lipidiques (*l*), les ovocytes (*o*), l'organe copulateur mâle (*com*), et la région postérieure vacuolisée (*v*).

(B) Partie postérieure, coupe sagittale, colorée par l'hématoxyline ferrique, montrant le pore génital mâle (*gpm*), l'organe copulateur mâle (*com*), et la région postérieure vacuolisée (*v*).

Frontal gland (Fig. 7C) prominent. Rhabdoids (Fig. 7A) abundant, in rather distinct rows, many of which converge at anterior end. Cilia about 7 μm long. Stiff sensory cilia, along margins of body, 19 μm long. Mouth (Fig. 7C) slightly anterior to middle of body. Posterior portion of body lacking conspicuous vacuoles characteristic of many acoels, including some other species of Otocelididae.

Common genital pore (Figs 7A-C, 8) on ventral surface close to posterior end. Seminal vesicle (Fig. 7A-C), enclosing eversible copulatory organ, anterior to genital pore. Sperm developing in lateral packets on both sides of body, beginning not far posterior to level of statocyst. Sperm channels (Fig. 7A, B) narrow but distinct, entering seminal vesicle on both sides. Sperm slender, approximately 125 μm long, 1.2 μm thick at end of first one-fifth of length.

Vagina (Fig. 7B, C), evident in compressed specimens as nearly hyaline structure, arching anterodorsally over seminal vesicle to seminal bursa (Fig. 7A-C). Nozzle of bursa 20 μm long, slightly curved, directed ventrally in undistorted specimens.

Remarks. *Otocelis rubropunctata* was abundant in the Punic Ports when I collected in these lagoons. It was easily found in sediment washed from *Ulva* and *Enteromorpha*, as well as in the sediment on the substratum beneath these algae.

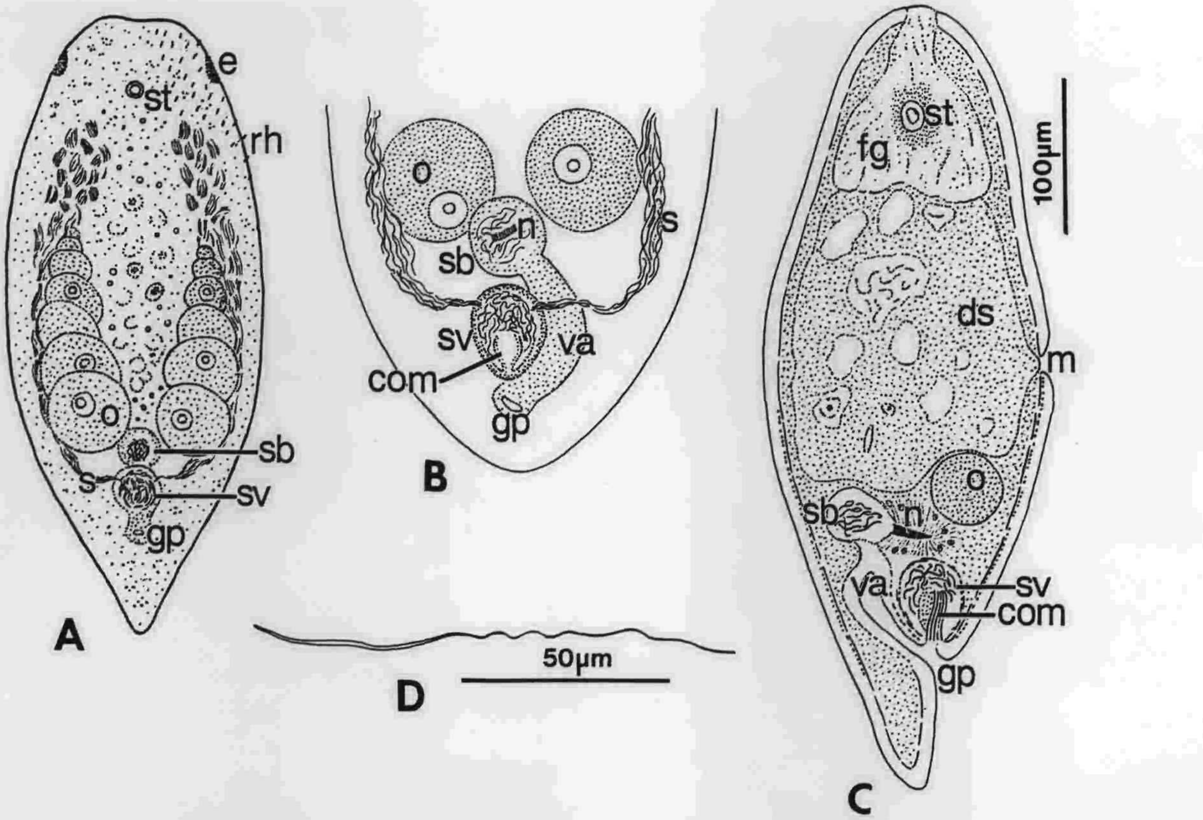

Figure 7. *Otocelis rubropunctata* (O. Schmidt).
(A) Living specimen, dorsal view. *(e)* eyespot, *(gp)* common genital pore, *(o)* oocyte, *(rh)* rhabdoids, *(s)* sperm channel, *(sb)* seminal bursa, *(st)* statocyst, *(sv)* seminal vesicle.
(B) Reproductive structures of living specimen, somewhat distorted due to flattening. *(gp)* common genital pore, *(n)* nozzle of seminal bursa, *(o)* oocyte, *(s)* sperm channel, *(sb)* seminal bursa, *(sv)* seminal vesicle, enclosing sperm and male copulatory organ, *(va)* vagina.
(C) Composite drawing based on sagittal sections stained with iron hematoxylin, prepared with the aid of a camera lucida. *(ds)* digestive syncytium, *(fg)* frontal gland, *(m)* mouth, *(st)* statocyste, others letters as in A and B.
(D) Living sperm, in fresh smear.
Figure 7. *Otocelis rubropunctata* (O. Schmidt).
(A) Spécimen vivant, vue dorsale. *(e)* tache oculaire, *(gp)* pore génital commun, *(o)* ovocyte, *(rh)* rhabdoïdes, *(s)* canal spermatique, *(sb)* bourse copulatrice, *(st)* statocyste, *(sv)* vésicule séminale.
(B) Appareil génital d'un spécimen vivant, un peu déformé par la compression, *n* canule de la bourse copulatrice, *sv* vésicule séminale, entourant les spermatozoïdes et l'organe copulateur mâle, *(va)* vagin, les autres abréviations comme en A.
(C) Reconstruction d'après des dessins à la chambre claire de coupes sagittales, colorées par l'hématoxyline ferrique, *(ds)* syncytium digestif, *(fg)* glande frontale. Les autres abréviations comme en A et B.
(D) Spermatozoïde vivant, dans un frottis frais.

Although some records for *O. rubropunctata* need confirmation, the wide distribution of this species is well documented. It is definitely known from the Black Sea and Bosporus, Adriatic Sea, northern Mediterranean, and North Atlantic.

This acoel was thoroughly described and/or illustrated by Graff (1891, 1904). Both accounts are supported by illustrations that collectively present a clear picture of its general appearance and the morphology of its reproductive system. Most of Graff's illustrations, together with scattered references to the morphology of *O. rubropunctata*, were published again in his review of the Turbellaria (1904-08). Brinkmann (1905) and Dörjes (1968) also studied the structure of this species, and in most respects their findings agree with those of Graff. In Dörjes' drawing of a living animal, however, the shape is not typical, and the areas on both sides of the body where sperm originate and mature are diagrammatically condensed. The acoel that Westblad (1946, 1948) identified as *O. rubropunctata*, and that he used as a basis for deciding which other species should be

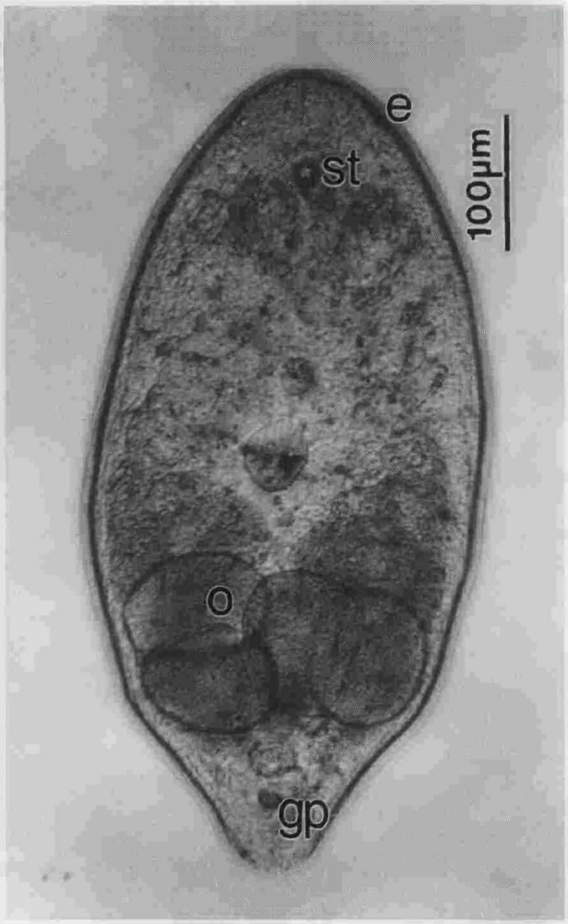

Figure 8. *Otocelis rubropunctata* (O. Schmidt), photomicrograph of living specimen, ventral view. *(e)* eyespot, *(gp)* common genital pore, *(o)* oocyte, *(st)* statocyst.

Figure 8. *Otocelis rubropunctata* (O. Schmidt), photomicrographie d'un spécimen vivant, vue ventrale. *(e)* tache oculaire, *(gp)* pore génital commun, *(o)* ovocyte, *(st)* statocyste.

included in the genus *Otocelis*, is different. Ax (1959), after finding worms that fit older descriptions of *O. rubropunctata*, proposed the name *O. westbladi* for the one studied by Westblad. Dörjes (1968) also observed *O. westbladi*, and his description confirms that the judgment of Ax was correct.

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