Three New Species of *Micrura* (Nemertea: Heteronemertea) and a New Type of Heteronemertean Larva from the Caribbean Sea

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ABSTRACT.—**Three new species of *Micrura* are described from shallow water of the Belize barrier reef and Bahia Almirante, Panama. Specimens were found at 0-30 m depth in association with a variety of rubble and its encrusting epibiota. The three species are morphologically very similar to each other, and are distinguished here primarily by color and by substantial differences in nucleotide sequence data for 16S rDNA and 28S rDNA. As many *Micrura*, they are slightly compressed dorso-ventrally, possess a caudal cirrus, and the cephalic lobe is bluntly rounded anteriorly and is the same width as the body. At least one species has distinct oblique musculature between the body-wall circular and inner longitudinal musculatures. One of the three species possesses a new morphotype larva rather than the expected typical pilidium larva.**

KEYWORDS.—**Nemertea, Taxonomy, Pilidium, Direct development.**

INTRODUCTION

Nemerteans are unsegmented, protostome worms characterized by an eversible proboscis housed in a mesodermally derived coelomic cavity, the rhynchocoel. They are common worms in most marine biotopes, but they often go unnoticed because of their cryptic habits. Ten papers have recorded a total of about 57 nemerteans from the Caribbean Sea and adjacent waters (Gulf of Mexico and Atlantic Ocean off southern Florida), 24 of which are heteronemerteans. Only 14 heteronemerteans have been recorded from the Caribbean Sea proper (Table 1), and there are no published records of heteronemerteans from waters off Belize or Caribbean Panama. However, our limited sampling of the latter two regions in recent years has revealed about 12 probable named species and up to 60 species of shallow-water nemerteans (unpubl. obs.). Here we describe as new three of the heteronemerteans, based on traditional morphological data supported by nucleotide sequence data.

The approximately 400 species of Heteronemertea plus the paleonemertean genus *Hubrechtella* comprise the Pilidiophora, for which the defining synapomorphy is the pilidium larva (Thollesson and Norenburg 2003). Usually, the pilidium is a more or less helmet-shaped, ciliated, planktonic and planktotrophic larva. However, direct-developing larvae have been described for three heteronemertean species, each with a unique type of larva and showing evidence of being a modified pilidium body bearing an encapsulated developing worm. Here we introduce, from one of the three new species, a fourth type of modified pilidium with encapsulated juvenile.

MATERIALS AND METHODS

Specimens were collected in the vicinity of the Smithsonian Institution's laboratory on Carrie Bow Cay, Belize in February 2000 and June 2002, as well as the Smithsonian Tropical Research Institute laboratory at Bocas del Toro, Panama, in August 2003. The following descriptions are based on observations of approximately 25 living specimens, and two sets of histological slides.

TABLE 1. Species reported from the Caribbean Sea.

Order	Species	Reference	Recorded Distribution
Palaeonemertea			
	Carinoma caraibica	Stiasny-Wijhoff, 1920	Curaçao
Heteronemertea			
	Baseodiscus antillensis	Bürger, 1895	Barbados
	Baseodiscus delineatus	Verrill, 1900	Bermuda
		Coe, 1901	Puerto Rico
		Stiasny-Wijhoff, 1920	Curaçao
		Coe, 1951a	Europe, Pacific North America Southern Florida, West Indies
		Corrêa, 1961	Southern Florida
	Baseodiscus discolor	Coe, 1901	Puerto Rico
	Baseodiscus unicolor	Stiasny-Wijhoff, 1920	Curaçao
	Cerebratulus antillensis	Coe, 1901	Puerto Rico
	Cerebratulus leucopsis	Coe, 1901	Puerto Rico
		Stiasny-Wijhoff, 1920	Curaçao
		Corrêa, 1961	Southern Florida
	Corsoua kristenseni	Corrêa, 1963	Curaçao
	Diplopleura curacaoensis	Stiasny-Wijhoff, 1920	Curaçao
	Dushia atra	Stiasny-Wijhoff, 1920	Curaçao
		Coe, 1951a	Southern Florida, West Indies
		Stiasny-Wijhoff, 1920	Curaçao
		Harris*, Pers.comm.	British Virgin Islands
		Corrêa, 1963.	Curaçao, Southern Florida
		Norenburg, Pers. obs.	St. Bathelemy
	Evanlineus tigrilis	Corrêa, 1954	Brazil
		Harris*, Pers. comm.	British Virgin Islands
		Pers. obs.	Southern Florida
	Tenuilineus albocinctus	Verrill, 1900	Bermuda
		Coe, 1901	Puerto Rico
		Corrêa, 1961	British Virgin Islands, Southern Florida
		Harris*, Pers. comm.	British Virgin Islands
		Schwartz, Pers. obs.	Belize, Caribbean Panama
	Micrura curacaoensis	Stiasny-Wijhoff, 1920	Curaçao
	Micrura vanderhorsti	Stiasny-Wijhoff, 1920	Curaçao
	Neolineus divae	Santos, 1974	Brazil
		Harris*, Pers. comm.	British Virgin Islands
		Schwartz, Pers. obs.	Pacific Panama, Southern Florida
Hoplonemertea			
	Amphiporus texanus	Coe, 1951a,b	Gulf of Mexico, Southern Florida
		Corrêa, 1963	Curaçao
	Carcinonemertes immunuta	Coe, 1951a	Gulf of Mexico, Southern Florida, West Indies
	Curranemertes natans	Kirsteuer, 1973	Venezuela
	Paradrepanophorus crassus	Coe, 1901	Puerto Rico
		Coe, 1951a	Southern Florida, West Indies
	Polyschista curacaoensis	Stiasny-Wijnhoff, 1920	Curaçao
	Poseidonemertes caribensis	Kirsteur, 1974	Bahamas, Belize, Jamaica, Caribbean Panama
		Bürger, 1895	Barbados
	Tetrastemma enteroplecta	Corrêa, 1954	Brazil
		Corrêa, 1963	Curaçao, Southern Florida, Brazil
	Tetrastemma herthae	Corrêa, 1963	Curaçao
	Tetrastemma merula	Corrêa, 1954	Brazil
		Corrêa, 1963	Curaçao, Southern Florida, Brazil
	Tetrastemma worki	Corrêa, 1961	Curaçao, Southern Florida
	Zygonemertes virescens	Coe, 1951a	Atlantic North America, Gulf of Mexico, Pacific North America
		Corrêa, 1963	Curaçao

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Dead coral rubble encrusted with epibiota was collected by SCUBA or snorkeling from shallow infralittoral to 30 m depths. These benthic samples were spread out in large bins filled with seawater and allowed to deoxygenate for up to two days (Kirsteuer 1967). Worms migrated to the edges of bins or to the top of the samples from where they were easily captured with a pipette. Animals were maintained for up to a week in shallow Petri dishes filled with seawater.

Specimens were annotated and photographed while alive, relaxed by drop-wise addition over 3-10 h of a 7.5% wt/vol solution of $MgCl_2$ mixed 1:1 with filtered seawater, fixed in seawater-buffered 10% formalin, and 1-2 weeks later transferred to Hollande-Bouin's (cupric picro-formol-acetic) fluid and/or 70% ethanol. Specimens for histology were dehydrated in a graded series of ethyl alcohols, cleared with multiple changes of xylene, and embedded in 56°C mp paraffin wax. Specimens of two species were sectioned and studied histologically. Samples were sectioned at 8 μm, mounted on slides, and stained with Crandall's polychrome method (Crandall et al. 1998).

For developmental study, gametes were liberated by cutting the body wall and the oocytes fertilized with sperm diluted in seawater (1/1000). Larvae were reared and maintained in finger bowls at room temperature (ca. 23-26°C).

Nemertean species previously recorded from the Caribbean Sea are listed in Table 1. The species collected during the present study are illustrated in the Photographic Identification Guide (this volume).

DNA was isolated and sequence data obtained and analyzed as in Thollesson and Norenburg (2003). Sequences for 16S rDNA and 28S rDNA are deposited with Genbank (Table 2).

SYSTEMATICS

Pilidiophora
Heteronemertea
Lineidae
Micrura Ehrenberg, 1828

TABLE 2. GenBank accession numbers.

	16S rDNA	28S rDNA
Micrura rubramaculosa	DQ022550	DQ022552
Micrura chlorapardalis	DQ022551	DQ022554
Micrura ignea	DQ022549	DQ022553

Diagnosis

Modified from Gibson (1981): single pair horizontal lateral cephalic slits; proboscis unbranched, with two or three muscle layers, and zero, one or two muscle crosses; rhynchocoel circular musculature not interwoven with body-wall musculature; fibrous core of dorsal ganglia bifurcates into upper and lower branches; neurocord cells absent; ganglia cell layer of brain not enclosed by neurilemma; foregut with or without splanchnic musculature composed of either circular or longitudinal muscles; caudal cirrus present; eyes present or absent; gonochoric.

Micrura rubramaculosa sp. n.

Etymology

The specific epithet refers to the leopard-like red mottling of the dorsal surface.

Specimens

The holotype, a sectioned female (a set of 8 slides, USNM 1081567); paratypes: one sectioned specimen (a set of 3 slides, USNM 1081573), and four whole specimens plus one anterior section in alcohol (USNM 1081568-1081572); deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC.

Localities

The type locale is shallow habitat around Carrie Bow Cay, Belize (16°48'N, 88°05'W). Worms were collected from coral rubble at 0-30 m depth. Hiding places on this rubble included cracks and crevices, encrusting marine invertebrates, and holdfasts of *Halimeda* sp. and coralline algae. Additional specimens were collected from similar infralittoral sites around Bocas del Toro field station, Panama.

External features

The largest specimens were 2.0 cm long, but most worms were approximately 1.0-1.5 cm, and 0.5 mm in width when fully extended and gliding (Fig. 1F). The ventral surface and ground color is opaque white to creamy white with red to red-brown mottling in more or less longitudinal streaks on most of the dorsal surface. The
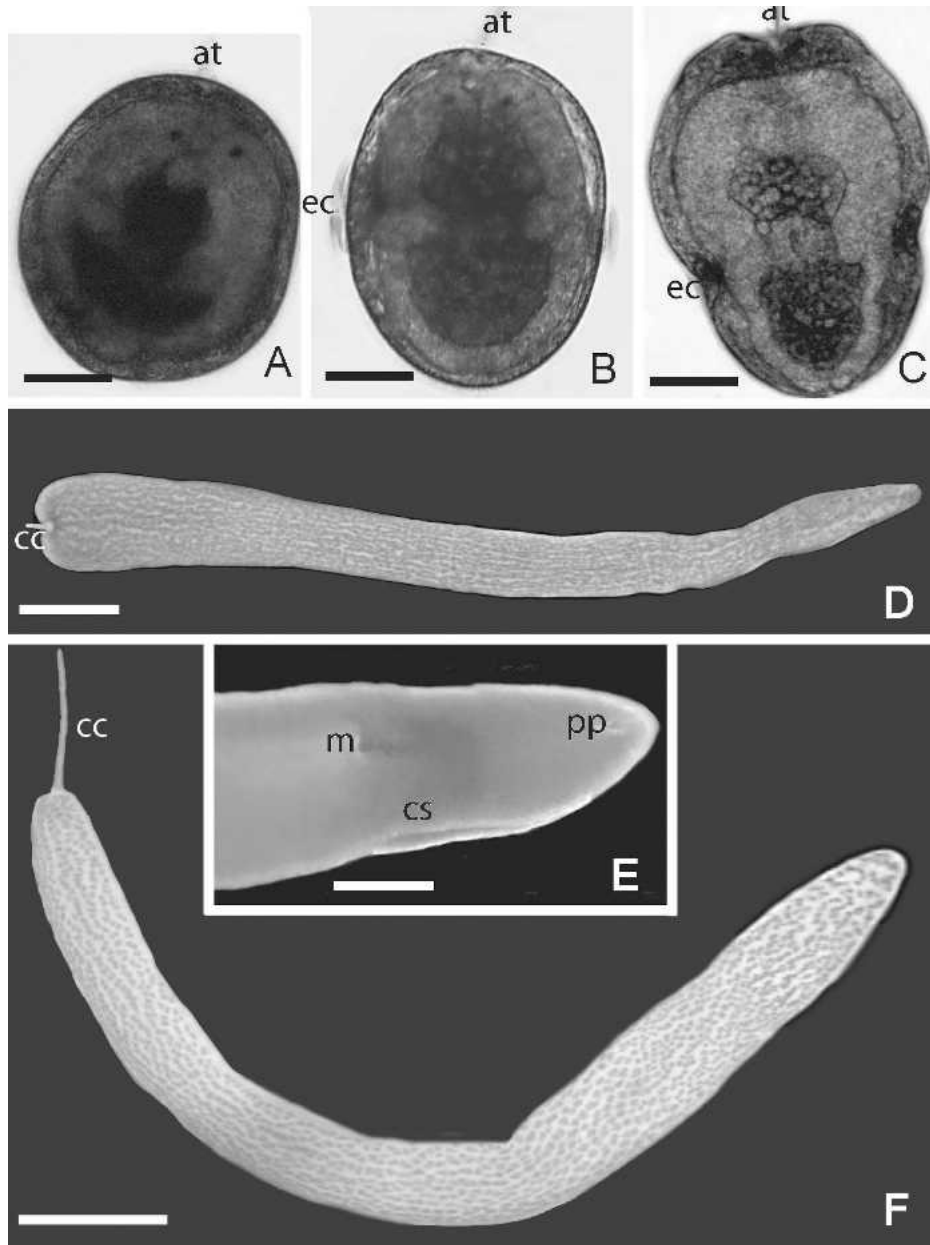

FIG. 1. A-C) *Micrura rubramaculosa* sp. n. A) 36-h larva. Scale = 30 μm. B) 72-h larva with equatorial ciliated band and apical tuft. Scale = 30 μm. C) Fully formed juvenile within larval body. Scale = 30 μm. D) *Micrura chlorapardalis* sp. n., adult. Scale = 4.0 mm. E) *Micrura ignea* sp. n., anterior end of adult. Scale = 1.5 mm. F) *Micrura rubramaculosa*, adult. Scale = 2.0 mm.

head is slightly spade-shaped and not distinct from the rest of the body except for a slight constriction at the posterior of the cephalic slits when the animal is creeping. At rest, the body is cigar-shaped and quite stout. When the worm contracts, the head is distinctly demarcated from the rest of the body by the posterior ends of the slits and the body becomes broad and flattened. The snout is slightly rounded and apical organs are present in a typical triangular pattern, with one mid-dorsal, and two ventro-lateral papillae. The papillae and their elongated cilia are obvious while the animal is creeping, and very sensitive to mechanical stimuli. The pink cerebral ganglia are visible through the dorsal and ventral body wall. The lateral horizontal slits are long and moderately shallow. Their interior surface is wide open to the environment at the anterior and posterior ends but the mid-region of the slits generally is compressed. Several groups of black ocelli are apparent within and throughout most of the length of the slits. The ocelli are more or less in rows, two rows on the margin of the upper lobe of each furrow, and a single row on the ventral lobe. The proboscis pore is positioned subterminally. The mouth is a small slit just posterior to the end of the cephalic slits. The animal is generally flattened and wider in the intestinal region. Pink ovaries of the female and white testes of the male, form rows on either side of the midline, and are visible through the body wall in the intestinal region. Paired gonopores are present on the dorso-lateral surface, near the lateral margins of the body, and beginning in the intestinal region. The posterior ends bluntly and bears a distinct caudal cirrus, situated dorsal to the anus. These worms have a very good capacity to regenerate; the caudal cirrus and posterior region were easily re-grown within 36 h of being removed.

When disturbed the worms either begin to swim with dorso-ventral undulations, or they contract in violent backward peristaltic motions. Occasionally, the contractions are violent enough to cause the worm to fragment.

Internal features

Body wall.—The epidermis is uniform in thickness and dominated by densely packed goblet cells (Fig. 2A). The goblet cells stain red with homogenous azanophilous serous material and have necks that occasionally bifurcate. Cyanophilous glandular cells are also present in the epidermis, but at a lower concentration than the serous cells. The goblet cells are separated by ciliated cells, which are attenuated basally and broad at the apex. The epidermis rests upon a thin basal lamina. Ciliated cells predominate and substantially fewer goblet cells are present in the epidermis of the cephalic slits (Fig. 2E). Glandular cells in the caudal cirrus are spherical and only in the epidermis (Fig. 3F).

The pigment granules giving the red mottled appearance to the dorsal surface are located in the dermis, adjacent to the basal lamina (Fig. 2A). Below the basal lamina, is a dermal zone (=cutis) as thick as or thinner than the epidermis. A very thin, barely perceptible, dermal circular muscle layer lies adjacent to the basal lamina and overlies a dermal longitudinal muscle layer that is 1-3 bundles thick, depending upon the state of contraction (Fig. 2B). These muscle layers overlie and are almost entirely separate from a deeper stratum of dermal (=insunk) gland cells. Most of these stain homogeneous red and discharge their contents through thin necks that penetrate the basal lamina and epidermis (Fig. 2A). Below the dermal tissue, is a well-developed (50.0 μm) main body-wall outer longitudinal musculature (OLM), followed by the lateral nerve cords and nerve plexus (Fig. 2B), a well-developed middle circular musculature (MCM) (20.0 μm) and inner longitudinal musculature (ILM) (10.0 μm) (Fig. 2B, 3A). Dorso-ventral muscles are not present.

Precerebral cephalic musculature consists of a meshwork of outer longitudinal muscle fibers mingled with radial and oblique muscle fibers between which various gland cells are distributed (Fig. 2D). The OLM of the body wall extends to the apex of the head, with some fibers turning obliquely outward. The radial fibers extend
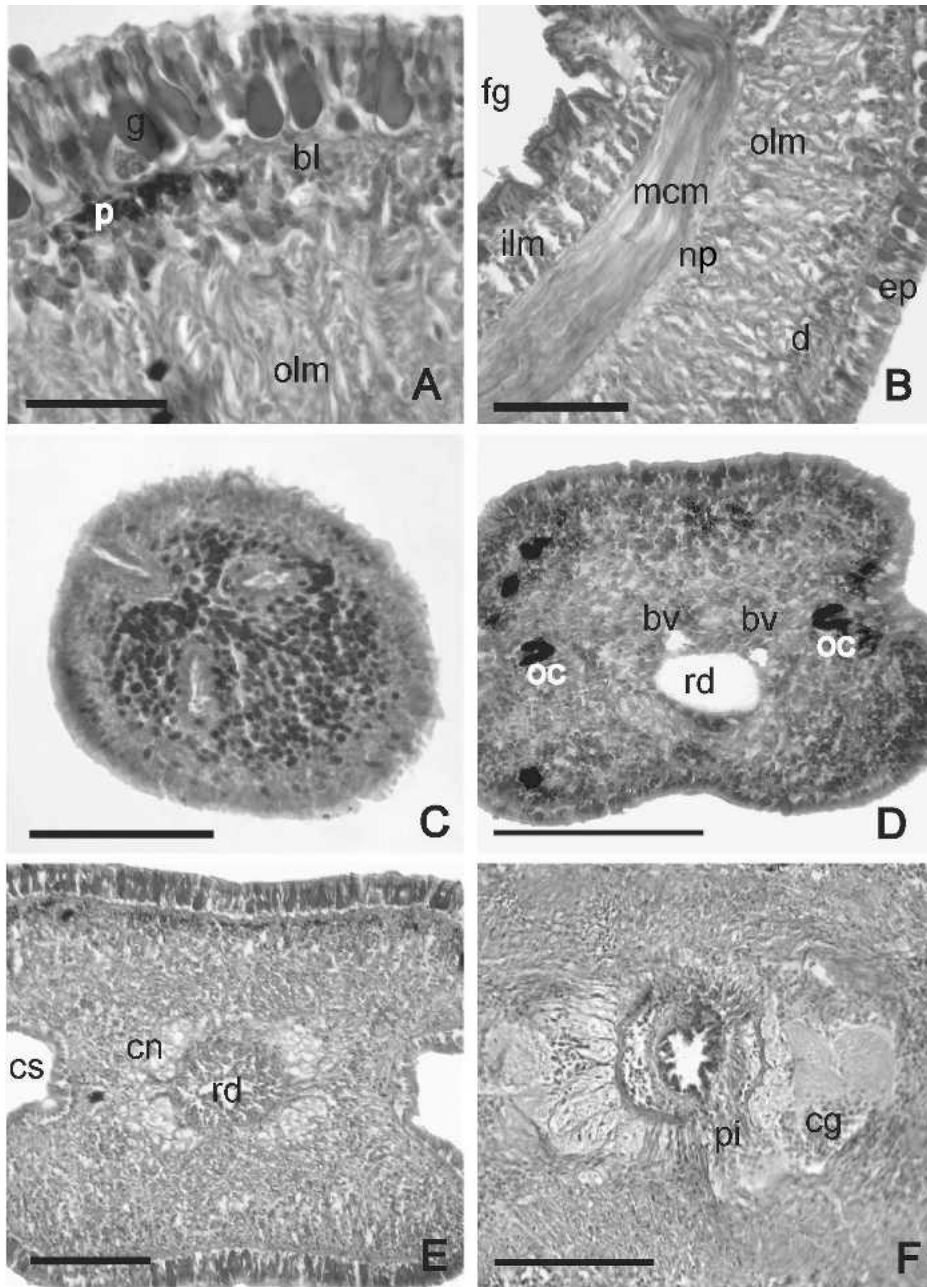
Fɪɢ. 2. A-F) *Micrura rubramaculosa* sp. n., photomicrographs. A) Epidermis with goblet cells, ciliated cells and necks of mucoid and serous cells of the dermis. Scale = 10 μm. B) Body wall. Scale = 50 μm. C) Inverted apical organs. Scale = 50 μm. D) Ocelli, rhynchodeum, and two cephalic blood vessels. Scale = 100 μm. E) Cephalic nerves and rhynchodeum. Blood vessels are barely visible due to animal contraction. Scale = 100 μm. F) Part of the proboscis insertion. Scale = 50 μm.
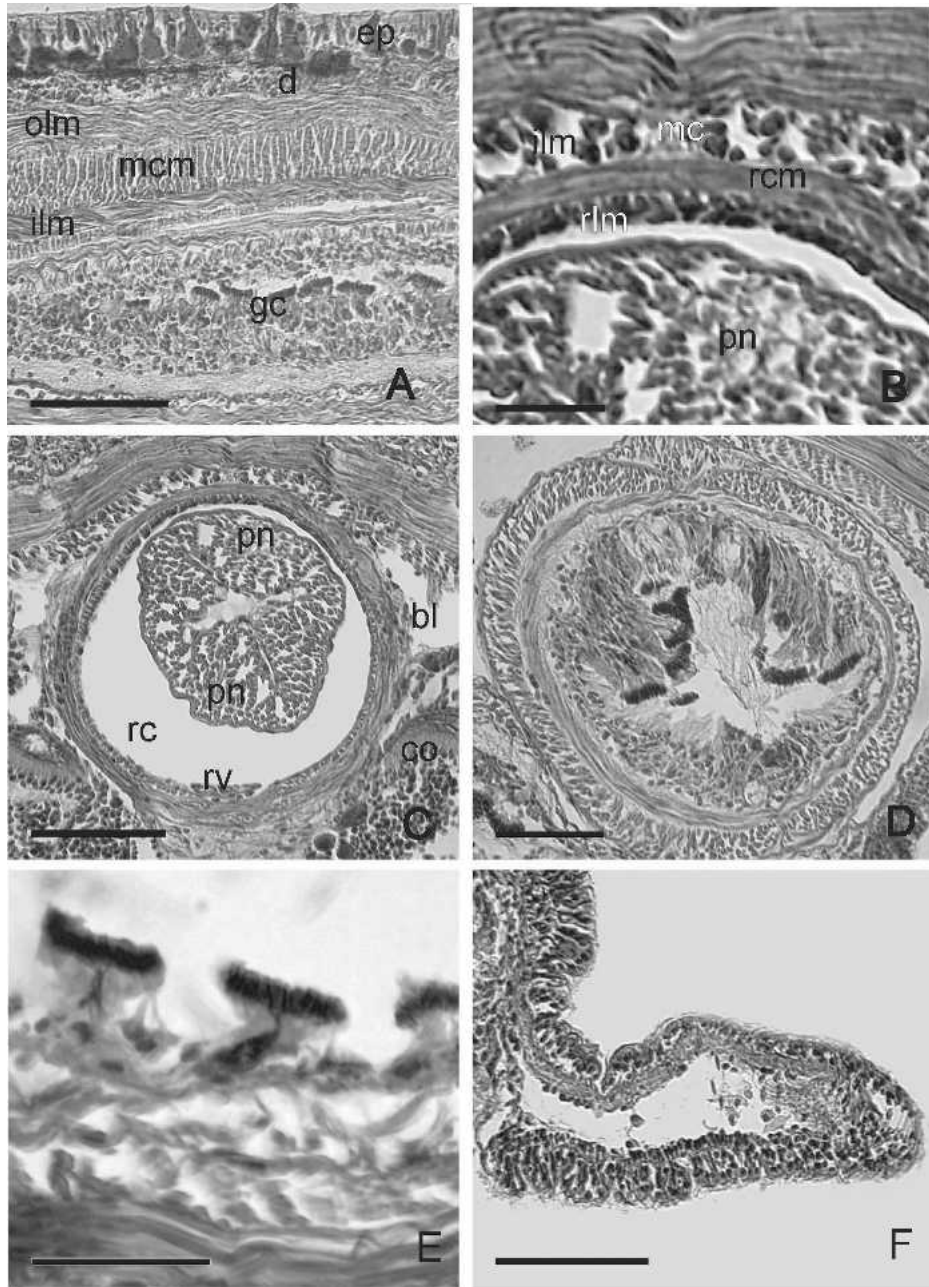
Fig. 3. A-F) *Micrura rubramaculosa* sp. n., photomicrographs. A) Frontal section of body wall and proboscis. Scale = 50 μm. B) Body-wall muscle cross. Scale = 10 μm. C) Anterior proboscis with proboscis nerves, and rhynchocoel villus. Scale = 25 μm. D) Posterior proboscis organization, note two muscle crosses. Scale = 25 μm. E) Proboscis glandular cups. Scale = 10 μm. F) Longitudinal section of caudal cirrus. Scale = 50 μm.

toward the cephalic slits and originate from or connect to the MCM, which encloses the rhynchodeum and cephalic blood lacuna (Fig. 2E) and forms a thick layer just anterior to the brain. Fibers originating from or connecting to the outer portion of the MCM invest the cerebral ganglia and the anterior portion of the cerebral organs, while other fibers extend out toward the epidermis. The MCM and the rhynchocoel circular muscle are in close apposition mid-dorsally throughout the length of the rhynchocoel. However, there are few instances where fibers seem to be crossing from one to the other (Fig. 3B). Posterior to the brain, the ILM is approximately two strands thick (Fig. 4B). The MCM is bisected by the esophageal nerves arising from the ventral ganglia. The anterior margin of the mouth appears a short distance posterior to the brain. Glandular cells of the foregut appear approximately 16 μm posteriad, internal to the MCM, which has been deflected upward by the mouth cavity. Alongside the opening of the mouth, dorsal-ventral muscle fibers reach from the dermis, through the ventral portion of the circular muscle and the glandular region of the foregut, and insert among the dorsal fibers of MCM. The inner longitudinal musculature is well-developed, approximately three or four fibers thick in this region.

Proboscis and rhynchocoel.—The rhynchodeum opens subterminally and ventrally from a shallow medial groove. Goblet cells and necks of subepidermal gland cells are absent from the epidermis of the proboscis pore furrow and the rhynchodael epithelium (Fig. 2D). The rhynchodeum is unciliated. The basal lamina surrounding the pore lumen is barely perceptible, and overlays a thin layer of dermal circular muscle over a relatively thick layer of dermal longitudinal muscle. Thick bundles of nerves, interrupted by radial muscle fibers, are just outside the longitudinal muscle bundles of the rhynchodeum (Fig. 2E). The proboscis insertion is just anterior to the cerebral ganglia and is laterally fenestrated to allow two cephalic blood vessels to pass through (Fig. 2F). The insertion fibers pass through the MCM, across the face of the cerebral ganglia, to the OLM. The proboscis is relatively thin anteriorly and a pair of proboscis nerves is evident here. More posteriorly, the main part of the proboscis is stouter, its epidermis is more differentiated, and the nerves become indistinct as they branch to form a plexus.

The proboscis is unbranched and possesses two muscle layers throughout its length—an outer circular musculature, separated from the glandular epidermis by the two proboscis nerves and associated nerve plexus, and a thicker, inner longitudinal musculature adjacent to the endothelium (Fig. 3D). Most of the proboscis epidermis is longitudinally differentiated into two types, with one forming a wide ridge that bears glandular, cup-shaped structures containing numerous, strongly staining, azanophilous, rod-shaped bodies at its apical surface (Fig. 3E). These derive from epidermal gland cells via a cell neck entering the base of each cup (Fig. 3E). Two approximately equally developed muscle crosses are present and extend from the circular muscle to the endothelium of the proboscis (Fig. 3D).

The rhynchocoel reaches almost to the posterior of the body. Rhynchocoel muscle fibers are not interwoven with the bodywall ILM (Fig. 3B.-C).

Vascular system.—Two thin-walled blood vessels flank the rhynchodeum and are joined anteriorly by a wide suprarhynchodeal connective (Fig. 2D). Posterior to the proboscis insertion, at the level of the brain commissures, these lateral vessels anastomose to form a U-shaped vessel cradling the ventral portion of the rhynchocoel. Toward the posterior of the brain, this vessel bifurcates and gives rise medially to the mid-dorsal blood vessel. Approximately 16 μm posterior to its origin, the mid-dorsal vessel forms a short rhynchocoelic villus approximately 360 μm long. For a short distance posterior to this, the mid-dorsal vessel widens and becomes bisected by connective tissue. At this point the lateral vessels are in a dorso-lateral position and widen to enclose the posterior portions of the cerebral organs before extending further posteriorly, adjacent to the rhynchocoel. The spacious anterior dorsolateral vessels each give rise to a ventrolat-
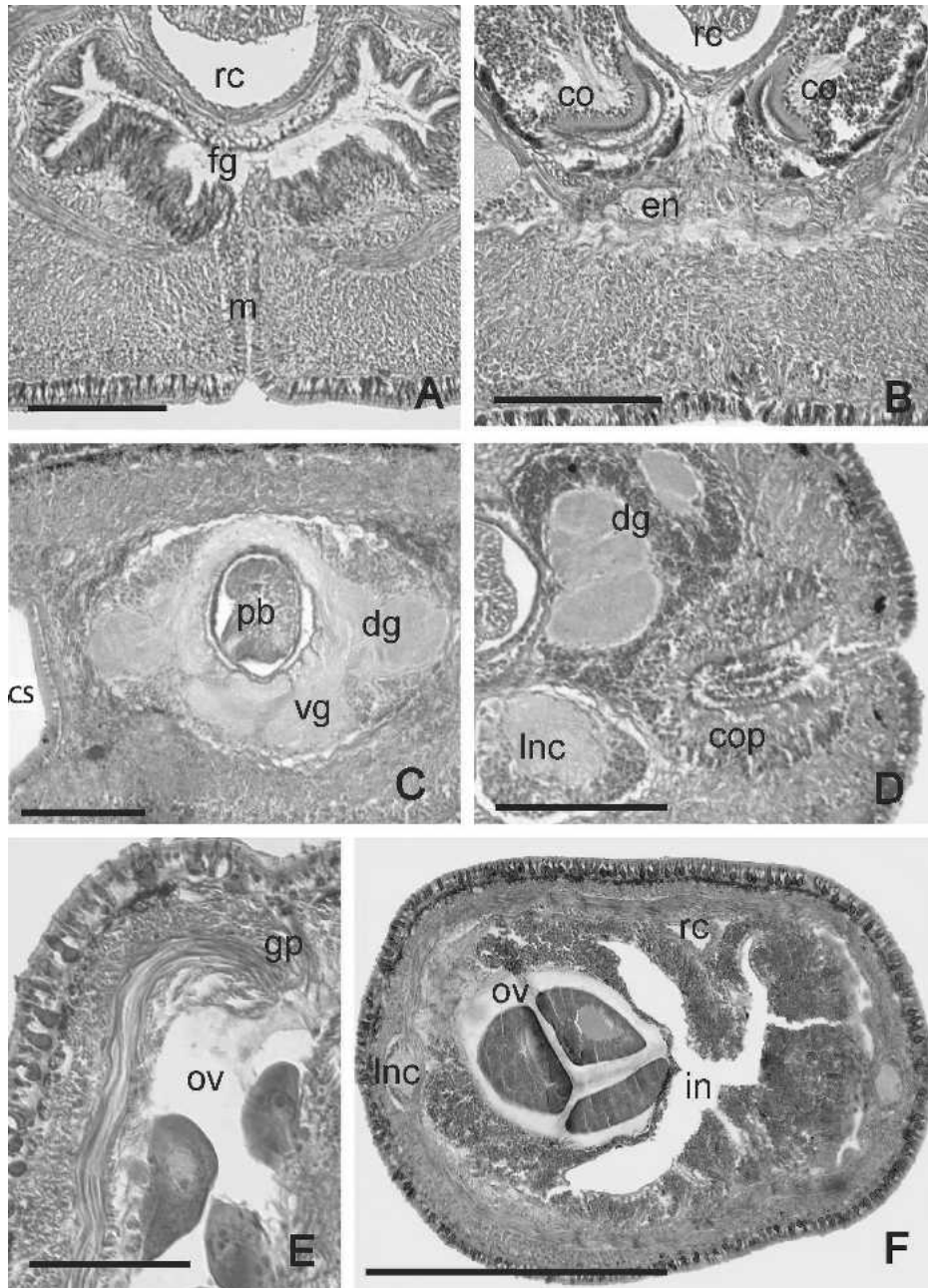
FIG. 4. A-F) *Micrura rubramaculosa* sp. n., micrographs. A) Mouth and beginning of the foregut. Scale = 50 μm. B) End of cerebral organ canals, and esophageal nerves. Scale = 50 μm. C) Dorsal lobes of cerebral ganglia connected by dorsal commissure and ventral lobes of cerebral ganglia. Scale = 50 μm. D) Posterior bifurcation of the fibrous core of dorsal ganglion and cerebral organ pit. Scale = 50 μm. E) Oogenesis and gonopore. Scale = 50 μm. F) Intestinal region and ovaries, one with three mature oocytes, and the other with numerous maturing oocytes. Scale = 200μm.

eral vascular plexus. The main vessels assume a lateral position along the foregut (Fig. 4A). The two lateral vessels and mid-dorsal vessel form a posterior anastomosis above the anus.

Digestive system.—The mouth is a short ventral slit posterior to the cephalic slits (Fig. 4A). The foregut's interior margin, or lip, is covered with columnar ciliated cells surrounded by numerous dermal gland cells. However, no specialized glands were observed and there were no apparent differences between the gland cells at the opening of the mouth and those of the foregut proper that could be considered specialized for salivation. The foregut wall is moderately convoluted with a conspicuous lining of cilia and microvilli.

The intestinal region has a convoluted wall with numerous large spherical gland cells and sparsely distributed ciliated cells. It is bounded externally by a discontinuous circular musculature (Fig. 4F). The anus is anterior to and below the caudal cirrus.

Nervous system.—The central nervous system comprises paired dorsal and ventral cerebral ganglia connected by transverse commissures and enclosed by connective tissue (Fig. 4C-D). The dorsal ganglia are larger than the ventral and are forked posteriorly into an upper and lower neuropil (Fig. 4D). The ventral commissure is much thicker (75 μm) than the dorsal (25 μm). The ventral ganglia are more elongate, extending for a short distance past the dorsal lobes, continuing posteriorly as the lateral nerve cords (Fig. 4D).

Numerous nerves extend forward from the dorsal lobes to innervate the head (Fig. 2E). A neural plexus encircles the body-wall middle circular muscle (Fig. 2B). A dermal dorsal nerve arises from the posterior of the dorsal commissure and proceeds posteriorly, adjacent to the body-wall middle circular muscle. A dorsal rhyncho-coelic nerve travels along the mid-dorsal contact zone between MCM and rhyncho-coel CM. Paired proboscis nerves originate at the dorsal commissure and enter the proboscis at its insertion. Two esophageal nerves arise from the inner lateral margin near the rear of the ventral ganglia and extend into the foregut wall (Fig. 4B).

Sense organs.—Three apical sense organs are located on the anterior tip of the head and arranged in typical heteronemertean fashion above the proboscis pore (Fig. 2C). These organs usually are everted papillae in life and apparent with a binocular microscope. However, with fixation they usually are retracted, so that they appear as pits in histological section. The epithelium of the organs is thin, densely ciliated, and lacks the more typical heteronemertean epidermal glandular cells. The pits are surrounded by dermal, spherical, glandular cells filled with a red homogeneous substance. These cells are confined to the anterior portion of the head and are interspersed between fibers of the cephalic musculature and discharge to the epidermal surface.

Numerous pigment-cup ocelli are arranged in longitudinal rows beginning at the apex of the head and continuing more or less as four rows inside each furrow, two on the dorsal and two on the ventral lobe of the furrow. Individual ocelli are 20 μm in diameter and are simple cups formed of small dark brown granules (Fig. 2D).

Lateral horizontal cephalic slits are moderately shallow and begin at the apex of the head and proceed up to the posterior margin of the cerebral organs. The cerebral organ canal opens into a pit anterior to the end of the slits (Fig. 4D). The neuroglandular cerebral organs abut the dorsal ganglia and are constructed as is typical of lineids. The epithelium of the cerebral organ canal bears very long cilia and lacks glandular cells, and is surrounded by nerve cells. Numerous large glandular cells surround, and their necks converge, on the blind terminus of the canal (Fig. 4B). A single cerebral organ nerve, originates from the mid-lateral face of the cerebral ganglia, near the cerebral organ canal, forks posteriorly, the lower branch turning ventrad and further inward as it wraps around the cerebral organ, while the upper branch seems to terminate in the circular musculature surrounding the brain.

Excretory system.—Small nephridial tubules extend along the two sides of the posterior half of the foregut, closely apposed to the lateral blood vessels and connecting to

a single duct, which leads posteriorly to an efferent canal, each opening dorsolaterally to a nephridiopore, near the posterior of the foregut (Fig. 5A).

Reproduction

The sexes are separate, with gonads distributed in dorsolateral rows on each side of the intestine. They are bounded externally by the inner longitudinal muscle layer of the body wall and internally by the circular muscle of the intestine (Fig. 4F) but separated from the muscles by well-developed connective tissue (Fig. 4E). Ovaries contain two to six mature oocytes, each about 130 μm in diameter, with a 50-μm germinal vesicle, and surrounded by a compact mucoid substance (Fig. 4F). Oviducts open externally through dorsolateral gonopores. Although active oogenesis was observed (Fig. 4E), most females appeared to be in a post-gravid state. Males were not observed in sectioned material.

Development

Upon contact with seawater, the oocyte's mucoid jelly coat plumps up and becomes spherical. The time to first and second cleavage is 1.0 h and 1.5 h, respectively. At about 36 h post-fertilization, a ciliated, spherical, swimming larva is formed, with a short apical tuft and two ocelli (Fig. 1A). After 72 h, the larva becomes elongated, the apical organ is anterior, and a band of opposing cilia encircles the long axis of the larva at midbody (Fig. 1B). By 96 h, the larva continues to swim and a fully formed juvenile is evident within the larval body (Fig. 1C). Although the larva and juvenile are opaque, it appears that development of the juvenile results from five blastodiscs, as is the case for Iwata's larva, but further corroboration is desirable.

Remarks

Micrura rubramaculosa fits the diagnosis for the genus *Micrura* as currently constructed (Gibson 1981, see above), except for the presence of several apparent dorsal rhynchocoelic muscle crosses (Fig. 3B).

However, this condition also is known from other accepted species of *Micrura*, including the type species, *Micrura fasciolata* (Cantell 1994, pers. obs.). Occurrence and structure of this muscle cross in other species needs further study.

Micrura akkeshiensis has a dorsal rhynchocoelic muscle cross, as well as a muscle cross in the proboscis. This species also possesses a modified pilidium larva, commonly referred to as Iwata's larva. The external features of Iwata's larva and that of *M. rubramaculosa* are very similar. However, the juvenile head within Iwata's larva is located 180° opposite to the larval apical plate, whereas it develops adjacent to the apical plate in the larva of *M. rubramaculosa*. The new larva also is unique in possessing an equatorial band of cilia about the larva's longitudinal axis. The potential homology of this ciliary band to the marginal band of a typical pilidium remains to be investigated.

Micrura chlorapardalis sp. n.

Etymology

The specific epithet refers to the green, leopard-like mottling of the dorsal surface.

Specimens

The holotype, a whole specimen (USNM 1081561); paratypes, five whole specimens in alcohol (USNM 1081562-1081563); deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC.

Localities

All specimens were collected from sites at Carrie Bow Cay, Belize (16°48′N, 88°05′W), the type locale. Worms were collected from coral rubble at 0-30 m depth. Hiding places on this rubble included encrusting marine invertebrates, cracks and crevices, *Halimeda* sp. and coralline algae holdfasts.
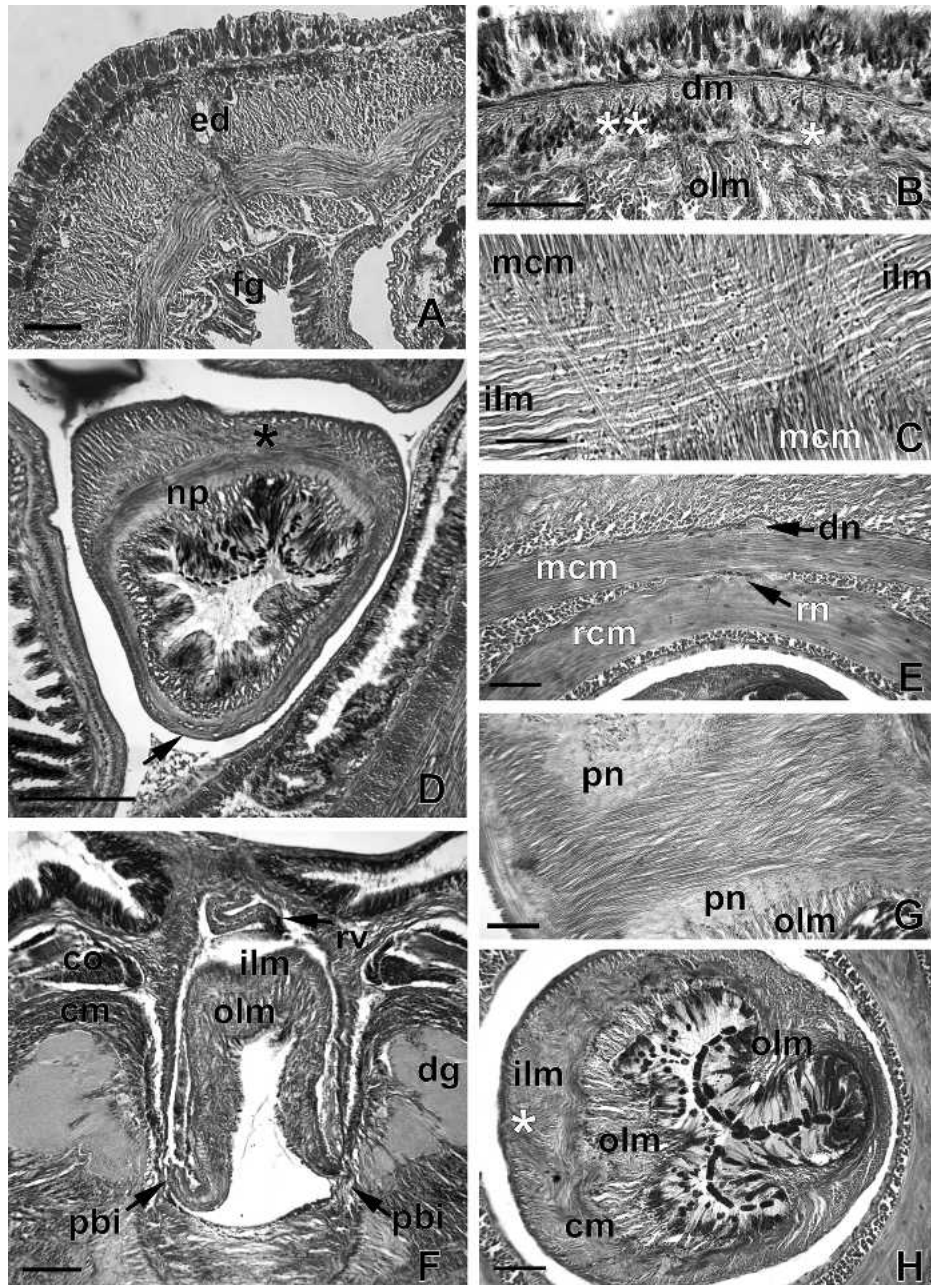
FIG. 5. A) *Micrura rubramaculosa* sp. n., micrograph. Transverse section through foregut region showing collecting tubule of excretory system and efferent duct. Scale = 50 μm. B-H) *Micrura ignea* sp. n., micrographs. B) Transverse section of proboscis, showing major muscle cross (*). Scale = 50 μm. C) Slightly oblique transverse section of proboscis, showing minor muscle cross (*) and major muscle cross (arrow). Scale = 200 μm. D) Transverse section of body wall with focus on epidermis and cutis (** = gland cell bodies; * = dermal nerve plexus). Scale = 50 μm. E) Tangential section through wall of proboscis, showing obliquely crossed fibers of minor muscle cross. Scale = 50 μm. F) Oblique frontal section through head, showing scarcity of proboscis insertion muscle. Scale = 100 μm. G) Transverse section through mid-dorsal body wall. Scale = 50 μm. H) Tangential section through body wall, showing oblique muscle fascicles between middle circular muscle and inner longitudinal muscle of body wall. Scale = 50 μm.

External features

Micrura chlorapardalis sp. n. is opaque white with faint pale-green to yellow-green leopard-like mottling streaked longitudinally over the dorsal surface, darker and heavier medially (Fig. 1D). It is similar in size to *M. rubramaculosa,* approximately 1.5 cm in length and 0.10 cm wide when creeping undisturbed. Ventrally, the worm is white with diffuse green mottling along the lateral margins and occasionally medially. The head is spade-shaped and widening slightly toward and then constricted at the posterior ends of the cephalic slits. The pink cerebral ganglia are visible through the dorsal body wall and located anterior to the end of the cephalic slits. The mouth is a short slit just posterior to the cephalic slits. The apical organ consists of three papillae arranged as in *M. rubramaculosa.* The body widens slightly posteriorly but is dorsoventrally compressed. The posterior end is blunt and bears an obvious, long, thin and very sticky caudal cirrus.

Remarks

The external features of *M. chlorapardalis* agree well with those of *M. rubramaculosa,* except for body color.

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Micrura ignea sp. n.

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Etymology

The specific epithet refers to the fiery-orange coloration of the entire animal.

Type specimens

The holotype, a whole specimen (four pieces plus ejected proboscis) in alcohol (USNM 1081564); paratypes, one frontally sectioned anterior body region on 13 slides (USNM 1081565), one specimen consisting of two midbody regions and hind end on three sets of slides (total = 45) and the anterior in paraffin (USNM 1081566); deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC.

Localities

The type locale is Cat Cay, Belize (16.66 N, 88.18 W). Additional specimens were col-lected from rubble at several sites near the Smithsonian marine laboratory at Bocas del Toro, Panama (09.15 N, 82.20 W), including Shark's Point, on the east side of Isla San Cristobal, a shallow flat with abundant coral rubble covered with filamentous turf algae, mat zoanthids, *Halimeda,* and *Thalassia.*

External features

Micrura ignea sp. n. is easily recognizable by its uniform bright orange color on the dorsal and ventral surfaces (Fig. 1E). Specimens were 1.0 to 2.5 cm long and 0.2 cm wide when creeping undisturbed. The cephalic lobe is pointed and widens toward the posterior end of the cephalic slits, at which a slight constriction demarcates it from the foregut region. The pink cerebral ganglia are visible through the cephalic slits and the body wall. The mouth is a small slit that begins posterior to the cephalic slits. The apical organ consists of three papillae arranged as in *M. rubramaculosa*. The body widens posteriorly and is dorsoventrally compressed. The posterior end is blunt and bears a conspicuous, thin and long caudal cirrus. These animals were found tightly wound inside a filamentous algal mat and fragmented quickly when stored in Petri dishes.

Internal features

Body wall.—The outer circular and inner longitudinal dermal musculature occupy the distal half of the cutis. Dermal glands occupy the basal half and are not separated from dermal LM but are separated from ILM by a thin, diffuse nerve plexus (there is no discrete zone of connective tissue, the dermis of some authors) (Fig. 5B). The MCM more or less ends at the posterior of the brain. There is a stratum of oblique muscle fascicles between MCM and ILM, one fiber thick, widely spaced, and orthogonally arrayed (Fig. 5C). A thin LM plate is present between foregut and rhynchocoel. Distinct dorsoventral musculature is lacking in the intestinal region.

Proboscis and rhynchocoel.—The rhynchodeal epithelium is poorly preserved, but

some cilia are evident. The proboscis epidermis is divided longitudinally into two regions, one a ridge bearing glandular cups with apical arrays of small rhabditiform granules (Fig. 5D, H), whereas the opposite epidermis appears to be raised into wide, transversely oriented papilliform folds bearing gland cells that lack rhabditiform granules. The pair of proboscis nerves entering the proboscis at its insertion gives rise immediately to a relatively distinct and uniform plexus (Fig. 5D, G). The proboscis musculature consists of three layers throughout. The proboscis ILM and OLM contribute weakly to the proboscis insertion (Fig. 5F), with few fibers extending into body-wall OLM across the face of the brain and others into body-wall ILM passing through the cerebral ring. The proboscis has one major (=standard) muscle cross between MCM and endothelium, and one minor muscle cross consisting of obliquely crossing MCM fibers that terminate in connective tissue fascia among ILM but do not reach the endothelium (the minor cross is evident only in oblique sections) (Fig. 5D, H). The major cross is opposite, and the minor cross is medially under the ridge epidermis. The rhynchocoel CM and body-wall MCM are distinctly separated by ILM in the foregut region. They are in contact dorsally throughout most of the body's remaining length (Fig. 5E), but with only sporadic and questionable instances of crossing muscle fibers along contact zone. There is no interweaving of rhynchocoel CM with body-wall ILM.

Vascular system.—Not fully characterized. A vascular plexus was not apparent. The paired lateral vessels are lacunar along the foregut, more thick-walled and muscular posterior to the foregut and lying ventrad to the main trunk of the intestine. The rhyncocoelic villus is approximately 300 μm long, distinctly tubular and lies in the rhynchocoel (Fig. 5F).

Digestive system.—The intestinal diverticula are poorly defined, and are demarcated primarily by gonads or muscle slings derived from rhynchocoel circular muscle passing under the intestine but not enclosing the lateral blood vessels. The foregut is diffusely enclosed in circular or transverse muscle fibers that extend radially outward at frequent intervals through ILM, apparently anchoring in MCM. Few, barely perceptible longitudinal muscle fibers surround the gut.

Nervous system.—There is no well-defined, so-called neurilemma around the ganglia or lateral nerve cords. The dorsal ganglia have a posteriorly bifid fiber core, forming two more or less laterally adjacent fiber cores. The dorsal nerve extends from the dorsal commissure forward into the head and backward over MCM for the full body length. A dorsal rhynchocoelic nerve is present between MCM and rhynchocoel CM (Fig. 5E), and communicates continuously with the dorsal nerve.

Excretory system.—Small, thick-walled nephridial tubules with a ciliated lumen are relatively abundant along the posterior half of the foregut, in close association with the vascular lacunae. Excretory pores were not seen.

Remarks

Internal anatomy of *Micrura ignea* is very similar to that of *M. rubramaculosa*, but some details of the anterior body (head through to anterior intestinal region) could not be reliably interpreted because only frontal sections were available. *Micrura ignea* closely resembles *M. rubramaculosa* and *M. chlorapardalis* in external features, except that it is bright orange; it differs from *M. rubramaculosa* in having a proboscis with three principal muscle strata and one distinct muscle cross, and a second, different kind of muscle cross. *Micrura ignea* also differs in micro-habitat and is not as hardy.

DISCUSSION

Micrura, with approximately 49 valid species, is the smallest of three "mega-genera" in the order Heteronemertea. However, there are no identified synapomorphies that distinguish it from the other two, *Cerebratulus* and *Lineus*. The three genera have character combinations that overlap and conflict among their alleged constituents. The conflicts are readily evident in cladistic analysis based on available morphological characters, in which each of the genera forms multiple para- and polyphyletic groupings (Schwartz and Norenburg

2001, unpub. obs.). However, there is a consensus of practice that *Micrura* species resemble those of the equally poorly characterized genus *Lineus*- the former with a caudal cirrus, the latter without one. Distinguishing *Micrura* from *Cerebratulus* is more problematic, as its species also have a caudal cirrus. *Cerebratulus* are alleged to have neurocords (a feature of the nervous system), whereas *Micrura* do not (Gibson 1982). Diagnosing *Micrura* species is difficult because their taxonomy is strongly biased toward internal anatomy, but this is unknown or insufficiently described for many species, even for the type species, *Micrura fasciolata* Ehrenberg 1828. Similarly, potentially important external features such as color and pattern are not known from life for many species. Furthermore, our own comparative studies lead us to be skeptical about the accuracy or validity of subtle internal characters alleged to diagnose many species based on single specimens, many of which were not or poorly relaxed prior to fixation. Thus, it is difficult and, at this time, simply impractical for morphological study alone to discern potential clades of *Micrura* species. The three proposed new species are treated together here in part because they are more or less sympatric, but mostly because, except for color, they strongly resemble each other in external appearance—in size, head and body-shape, and the conspicuously blunt posterior, which is not a common feature among heteronemerteans.

It is an article of faith in nemertean taxonomy that species descriptions require a full description of internal anatomy. This is desirable in the long run, to expand, correct and reinterpret phylogenetically the existing dataset. For instance, oblique body-wall musculature between MCM and ILM has been reported only rarely for heteronemerteans and interpreted as a relatively important finding. We expect, just as its homologue in hoplonemerteans was underreported for decades, that this oblique musculature is widely distributed among heteronemerteans, but recognition of it requires both anticipation and the appropriate sections. In the present work, we cannot clearly assess the apparent differences in proboscis muscle crosses between the two species examined. The implication seems to be that not all muscle crosses are the same and that recording only the number of crosses may be misleading. However, emphasis on cataloging internal anatomical characters, especially doing so critically, conflicts with the goal to document and inventory diversity expeditiously (see also Roe and Norenburg 1999). We are confident and comfortable that the three proposed species are adequately diagnosed on relatively superficial morphological criteria, even if no unambiguous internal anatomical diagnostics were to be forthcoming. Molecular data for two genes strongly substantiate our three species hypotheses. *Micrura ignea* is separated from *M. rubramaculosa* and *M. chlorapardalis* respectively by genetic distances of 22% and 30% for 16S rDNA, and 12% each for 28S rDNA. *Micrura rubramaculosa* and *M. chlorapardalis* are separated from each other by genetic distances of 24% and 2% for 16S rDNA and 28S rDNA respectively. These are large distances (pers. obs.). Nevertheless, the great morphological similarity of the three proposed new species is confirmed in a parsimony analysis of 83 heteronemertean species that places these three together in a clade by themselves (unpub. obs.).

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Appendix: Abbreviations used in figures

at	apical tuft
bl	basal lamina
blc	blood lacuna
bv	blood vessel
cc	caudal cirrus
cm	circular muscle
cs	cephalic slit
cg	cerebral ganglion
cn	cephalic nerves
co	cerebral organ
cop	cerebral organ pit
d	dermis
dg	dorsal ganglion
dm	dermal muscle
ec	equatorial ciliated band
ed	efferent duct
en	esophageal nerve
ep	epidermis
fg	foregut
g	goblet cell
gc	glandular cup
gp	gonopore
ilm	inner longitudinal muscle
in	intestine
lnc	lateral nerve cord
m	mouth
mc	muscle cross
mcm	middle circular muscle
np	nerve plexus
o	oocyte
oc	ocellus
olm	outer longitudinal muscle
ov	ovary
p	pigment
pb	probocis
pi	probocis insertion
pn	proboscis nerve
pp	proboscis pore
rcm	rhynchocoel circular muscle
rc	rhynchocoel
rd	rhynchodeum lumen
rlm	rhynchocoel longitudinal muscle
rn	rhynchocoelic nerve
rv	rhynchocoel villus
vg	ventral ganglion