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Systematics and taxonomy of polyclad flatworms with a special emphasis on the morphology of the nervous system

Sigmer Y. Quiroga
University of New Hampshire, Durham

SYSTEMATICS AND TAXONOMY OF POLYCLAD FLATWORMS WITH A SPECIAL

EMPHASIS ON THE MORPHOLOGY OF THE NERVOUS SYSTEM

BY

SIGMER Y. QUIROGA

BS, Universidad Jorge Tadeo Lozano, 2003

DISSERTATION

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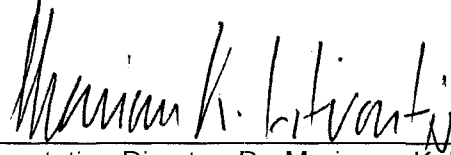
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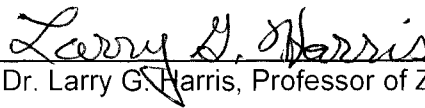
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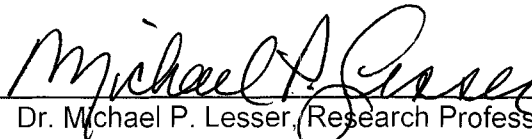
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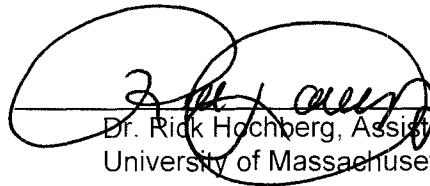
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DEDICATION

This work is dedicated to my mother who always worked really hard to give me the chance to complete my studies. She is my guide and example of how far the human being can go.

To the memory of my father who thought me how to keep the spiritual strength to stand the hard times in my life.

To Leila and Abril who illuminated my life at the right time.

"What caterpillar calls the end, the master calls a butterfly" Richard Bach.

ACKNOWLEDGEMENTS

PREFACE

When I was about to complete my undergraduate studies in Marine Biology, I decided to include a senior project on a little-known group of invertebrate worms. In a book on Invertebrate Zoology, my research colleague Marcela Bolaños and I had learned about polyclads flatworms, and were immediately attracted to their beauty and diversity. However, I was skeptical about finding such creatures in my home country, Colombia. Encouraged by rumors from some of our classmates about the existence of polyclads along the Colombian shores, we embarked on our first field trips. Amazingly, they were there, almost imperceptible, under rocks and coral rubble, gliding smoothly into crevices and hollows! Ever since then, I have not been able to stop working on this extraordinary and fascinating group.

Nestor Ardila, a marine biologist at INVEMAR in Santa Marta, Colombia assumed the challenge of directing our thesis, and from Australia, Dr. Leslie Newman was the only researcher working on polyclads who was willing to help us despite the distance. We collected flatworms for an entire year, and during that time, Dr. Newman introduced us to Dr. Marian Litvaitis who proposed we apply to the University of New Hampshire and enroll in the Master of Science program in the Department of Zoology.

After defending our work in Colombia, we arrived at UNH, where we completed the identification of all species we had collected along the Caribbean cost of Colombia. During that first year at UNH, we published a checklist of the polyclads from Colombia. This was followed by the description of a new species, *Armatoplana colombiana*. The

checklist of Colombian polyclads constitutes the second chapter of this doctoral dissertation.

In addition to the taxonomic part, a description of the nervous system of a locally abundant species of polyclads *(Pleioplana atomata),* was one of the main goals of my Master's research. Because my advisor secured funding from the National Science Foundation in 2004, my research goals expanded dramatically, and I changed my degree program to a PhD. During the NSF-funded project, we surveyed and collected polyclads flatworms throughout the Caribbean. The project is on-going and will result in several additional species descriptions and a key to the polyclads of the Caribbean. My project also involved museum work at the American Museum of Natural History and the National Museum of Natural History. Dr. Janet R. Voight from the Field Museum of Natural History of Chicago provided me with undescribed deep-sea species to be identified. As a result, I established a new family, a new genus and described two new species from the North Pacific, which are the focus of the third chapter of this dissertation. Two additional new species from the continental slope of the Gulf of Mexico comprise the fourth chapter, rounding out the taxonomic component of my dissertation.

Recognizing the need that new morphological characters are needed to elucidate relationships within the Polycladida, I focused my attention on the gross anatomy of the nervous system. The fifth chapter contains the results from the analysis of the central nervous system of several species.

This dissertation is the result of five years of research and is part of a larger team effort of the so-called "Flatworm Wranglers," an enthusiastic group of people, consisting of Marian Litvaitis, Leslie Newman, Marcela Bolaños, Kate Rawlinson, Marcin Liana, Anne DuPont and me, Sigmer Quiroga.

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ABSTRACT

SYSTEMATICS AND TAXONOMY OF POLYCLAD FLATWORMS WITH A SPECIAL EMPHASIS ON THE MORPHOLOGY OF THE NERVOUS SYSTEM

By

Sigmer Y. Quiroga

University of New Hampshire, September, 2008

In a first survey of the Colombian polyclad fauna, a total of 25 species were collected from the rocky littoral of the Tayrona National Park, Santa Marta, Colombia and deposited at the Museo de Historia Natural Marina del INVEMAR. Six species represented first records for Caribbean region. Furthermore, a new combination *Phrikoceros mopsus* nov. comb. was proposed, and a possible new species of *Pleioplana* Faubel, 1983 was found. In addition, a new polyclad family Anocellidae was erected and four deep-sea species were described; two species from the North Pacific Ocean, *Anocellidus profundus* gen. nov. sp. nov. and *Oligocladus voightae* sp. nov., and two from the continental slope of the Gulf of Mexico, *Oligocladus bathymodiensis* sp. nov. and *Didangia carneyi* sp. nov All except *D. carneyi,* were found in association with bivalves. The type material was deposited at the Field Museum of Natural History, Chicago, Illinois, USA. All taxonomic work was based on major external features and serial sagittal sections of the reproductive system. Finally, the central nervous systems (CNS) of 12 species of polyclad flatworms belonging to 11 families were examined using traditional light microscopic techniques. Even though some morphological features of the CNS probably were related to the body shape and behavior of the species, three categories could be established.

These categories were based on the presence and development of globuli cell masses, the cross-sectional shape of the main nerve cords, and the tissue type surrounding the nerve cords. Well-developed globuli cell masses characterize all acotylean species examined. Furthermore, the cotylean *Pericelis cata* also had well-developed globuli cell masses, providing additional evidence of the close phylogenetic relationship of Pericelidae with the Acotylea. Cotylean polyclads on the other hand, exhibit only weakly developed globuli cell masses. Unique features of the CNS were found in *Boninia divae* which represent autapomorphies for the family and which can be linked to the behavior and body shape of this taxon. The presence of external globuli cells masses in some polyclads, forming structures similar to arthopod mushroom bodies, may be an indication of an early evolutionary adaptation.

CHAPTER I

A GENERAL INTRODUCTION TO THE POLYCLADIDA (PLATYHELMINTHES)

Traditionally, the phylum Platyhelminthes was considered to be an early branch among the bilateral phyla (Hyman 1951). It consists of acoelomate, dorsoventrally flattened worms that lack circulatory and respiratory systems (Hyman 1951). Although Ehlers (1986) presented the following apomorphies for the taxon: a) absence of mitosis in somatic cells, i. e., somatic cells differentiate from blastomeres or stem cells in post-embryonic stages; and b) multiciliated cells, where cilia lack accessory centrioles. However monophyly of the group cannot at present, be established reliably.

Currently, deuterostomes and two groups of protostomes, Ecdysozoa and Lophotrochozoa are the three main clades of metazoans (Dunn et al. 2008). Recent advances in molecular systematics firmly place the phylum Platyhelminthes (minus Acoelomorpha) into the Lophotrochozoa (Dunn et al. 2008) (Fig 1A). This is supported by the presence of spiral cleavage, and probably by the fact that several flatworms have larvae that superficially resemble the trochophore larva of lophotrochozoans (Young et al. 2006). In contrast, other phyla belonging to this clade have a coelom, a one-way through gut, and some of them show segmentation. The reductions seen in today's flatworms are thought to be secondarily brought about by progenesis (Salo et al. 2001)

In other words, Platyhelminthes are derived lophotrochozoans and are no longer considered to be primitive bilaterians. In the past, the Platyhelminthes had been divided into the free-living Turbellaria, and the parasitic Trematoda, Monogenea, and Cestoda (Fig 1B). However, no autapomophies have been found that define the Turbellaria. Characters such as "free living" and "body covered by a multiciliated epidermis" have been shown not to represent defining characteristics for the taxon. Thus, the term "Turbellaria" currently is used in a descriptive way. Within the turbellarians, two groups can be recognized, namely micro- and macroturbellarians. These designations however, have no systematic value either; they are only used for size descriptions.

In a cladistic analysis, Ehlers (1986) divided the phylum into the Catenulida and the Euplatyhelminthes, the latter containing the Acoelomorpha and Rhabditophora. The Acoelomorpha consists of the orders Acoela and Nematodermatida, whereas the Rhabditophora comprises all other platyhleminth orders, including all parasitic forms. The position of the Acoelomorpha and even the validity of the taxon itself has been the subject of much debate (Ruiz-Trillo et al. 1999; Philippe et al. 2007). The latest data from molecular embryology further support the non-monophyly of Platyhelminthes (*sensu* Ehlers 1986). In fact, Acoela may even be more closely related to deuterostomes rather than Lophotrochozoa or protostomes (Cook 2004; Deutsch 2008) (Fig1A).

The order Polycladida (formerly considered in the "Turbellaria") is now included among the Rhabditophora, and based on the size of its species consists of macroturbellarians. An additional grouping includes the polyclads in the Archoophora together with the Catenulida, the Acoelomorpha, Macrostomida, and Haplopharyngida (Karling 1967, 1974). This grouping is based on an organizational grade derived from the homocellular arrangement of female gonads and the production of entolecithal eggs (Hyman 1951). This organizational grade contrasts with the Neoophora, which are

characterized by heterocellular gonads and ectolecithal eggs and include all remaining

rhabditophorans. Again though, both terms carry no systematic value and are best used

for developmental and reproductive descriptions only.
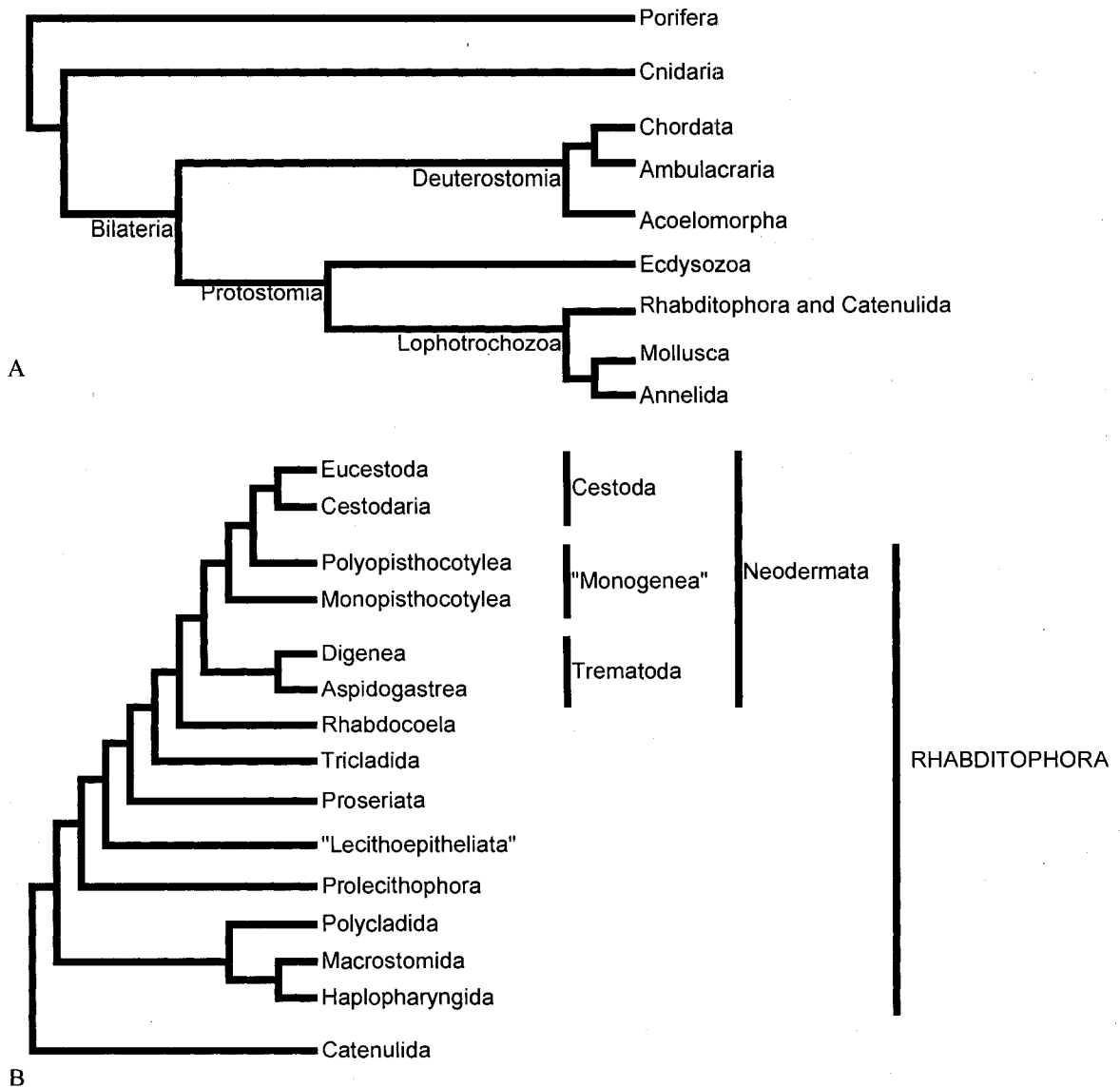
Figure 1 A. Phylogenetic tree of the metazoans (modified after Philippe et al. 2007) B. Phylogenetic tree of platyhelminth relationships (modified after Ehlers 1986). Changes from Ehlers' tree are as follows: Rhabdocoela does not include the neodermatan taxa; cestode relationships follow Hyman (1951); a paraphyletic Monogenea (Litvaitis and Rohde 1999), the inclusion of the orders Haplopharyngida, "Lecithoepitheliata", and Prolecithophora. Groups in quotation marks represent taxa of uncertain monophyletic status.

Polyclads are almost exclusively marine; only one species of the genus

Limnostylochus lives in freshwater (Hyman 1951). They commonly dwell on coral and

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rocky reefs, but they can even be found in hostile habitats such as in deep-sea environments (Quiroga et al. 2008, 2006). Although they are not parasitic, some of them live in association with other invertebrates, especially mollusks, crustaceans and echinoderms. Others can be found living in the intertidal zone, in empty mollusk shells, cavities of sponge beds, barnacles and bivalves (Prudhoe 1985). The main characteristic of the group is their highly branched intestine (Hyman 1951), from which they derive their name (poly = many; clade = branches). The presence of a highly ruffled pharynx known as a pharynx plicatus and the reabsorption of blastomeres are additional autapomorphies of the taxon (Ehlers 1986). Polyclads, like all platyhelminths, are hermaphrodites but do not self-fertilize. Their development can be direct or indirect from entolecithal eggs. Indirect development involves either a Götte's or a Müller's larva (Hyman 1951).

Polyclads have few external traits. However, the presence or absence of clusters of eyespots and either true tentacles or pseudotentacles, which are formed by folds of the anterior body margin, can be used as systematic characters (Newman and Cannon 1994b). The initial division of the order though, is based on the presence or absence of a ventral sucker. This character divides the polyclads into the two suborders Acotylea (without sucker) and Cotylea (with sucker) (Lang 1884).

The polyclad reproductive system is complex and has major importance for taxonomic identifications. However, current characters used for taxonomic separation lack significant phylogenetic value. As a result, only a few phylogenetic studies exist for polyclads (Litavitis and Newman 2001, Rawlinson and Litvaitis 2008). The most recent cladistic analysis of the Cotylea is based on morphology suggests their monophyly, and added additional synapomorphies to the taxon such as a short posteriorly extending vagina and presence of cement glands (Rawlinson and Litvaitis 2008). Molecular studies have

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supported the importance of the color patterns for the species identification among some

cotyelans, especially in the Pseudocerotidae (Litvaitis and Newman 2001). Molecular data

have also been shown to provide an important tool for the identification of polyclads flatworms

(Goggin and Newman 1996). In fact, the most recent species descriptions include molecular

sequence tags (Bolaños et al. 2007).

The worms are also of interest to scientists in the fields of toxicology and regeneration.

Studies have shown the presence of several toxic chemicals in their tissues, such as

staurosporine derivatives (Schupp et al. 1999), tetrodotoxin (Jeon et al. 1987; Miyazawa et al.

1987; Ritson-Williams et al. 2006) and neurotoxins similar to ciguatera (Newman and Cannon

2003). Additionally, some polyclads produce epidermal secretions that can kill other animals

(Laidlaw 1902) or, if injected into others organisms, can produce toxic symptoms sometimes

with lethal results. Polyclads show the fascinating ability of regeneration but compared with

freshwater triclads, their regenerative powers are relatively low. Fragments containing the

cerebral organ will regenerate into complete animals, whereas fragments posterior to the

cerebral ganglion are not able to regenerate the anterior end (Child 1904a, b, 1910; Olmsted

1922, Levetzow 1939). Most previous regeneration studies using flatworms have concentrated

on the planarian triclad *Dugesia* to the point where this worm has become a model organism

for the study of cellular differentiation and regeneration (Baguñà et al. 1994).

Finally, many acotyleans are active predators on commercial aquaculture species. For

example, some species of *Stylochus* devour eggs and spat of oysters (Pearse and Wharton

1938, Galleni et al. 1980, Newman and Cannon 1993), and are considered pests of

commercial bivalves including rock oysters, pearl oysters and giant clams (Jennings and

Newman 1996b).

CHAPTER II.

A CHECKLIST OF POLYCLAD FLATWORMS

(PLATYHELMINTHES: POLYCLADIDA) FROM THE CARIBBEAN COAST

OF COLOMBIA, SOUTH AMERICA[1]

Introduction

Members of the flatworm order Polycladida commonly dwell on coral and rocky

reefs, and may live in association with other invertebrates, especially mollusks, crustaceans

and echinoderms. The main characteristic of the group is a highly branched intestine

(Hyman 1951), from which they derive their name. Polyclads have few external taxonomic

characteristics and positive species identifications are mostly based on the structure of the

reproductive system. The initial division of the order into two suborders, namely the Acotylea

and the Cotylea, is based on the absence or presence of a ventral sucker, respectively

(Lang 1884). Additional external characteristics used for taxonomic identifications include

the presence and arrangement of eye spots (e. g., cerebral, tentacular, clustered, marginal),

the presence of either true tentacles or pseudotentacles (i. e., mere folds of the anterior body

margin), and the structure of the pharynx (Newman and Cannon 1994b). Among the cotylea

many species are conspicuously colored and exhibit striking color patterns. Hyman (1954a)

and Prudhoe (1985, 1989) both maintained that cotylean color patterns represent valid

systematic characteristics that can be used for taxonomic identifications. Newman and Cannon (1995) also have demonstrated the importance of color and color patterns in the identification of cotyleans. However, in most cases, and especially for acotyleans, unequivocal species identifications are based on serial sections of the reproductive system (Faubel 1983, 1984a).

The greatest number and diversity of genera and species of polyclads occur in tropical regions (Prudhoe 1985). But despite numerous species of tropical polyclads recorded (Hyman 1954b, 1955a, b, 1959a, b; Marcus 1960; Marcus & Marcus 1968; Prudhoe 1985; Jennings and Newman 1996a, b; Newman and Cannon 1994a, b, 1996a, b, 1997, 1998, 2000, 2002; Newman et al. 2003), their diversity is not well known because of difficulties in collecting, handling, and identifying specimens. Many of the earlier studies based their descriptions on single specimens that, in some cases, were immature, or on preserved specimens that were badly contorted due to improper fixation. Because of their extremely fragile nature and tendency to autolyse, polyclads are rarely collected intact and, as a consequence, they are inadequately represented in museum collections. Finally, any diagnostic color or color patterns fade and disappear rapidly after fixation, and thus, there is a great need for photographic documentation of living specimens, an approach initiated only recently with the work of Newman and Cannon (1994a, b, 1995).

Currently, two polyclad classification systems exist (Faubel 1983, 1984a; Prudhoe 1985). The classification system of Faubel (1983, 1984a) is based on the characteristics of the male reproductive system, specifically the structure of the prostatic vesicle and its orientation and relationship to the ejaculatory duct. The present checklist mostly follows the classification system proposed by Faubel (1983, 1984a) with the inclusion of the new genus Phrikoceros that had been proposed by Newman and

Cannon (1996a). Knowledge about Caribbean polyclads is limited to the works of Hyman (1955a, b), Prudhoe (1944), and Marcus and Marcus (1968), and is non-existent for the Caribbean coast of Colombia. This study represents the first such survey of Colombian polyclads, and therefore all specimens are first records for this area.

Collection Sites and Methods

Three collection sites (240 m^2 combined area), Punta de Betín, Playa Cristal, and Inca-Inca Bay were selected based on habitat features and accessibility. Punta de Betín (74° 13' W; 11° 15' N) is located in the northeast of Santa Marta Bay, forming a rocky peninsula that consists of small hills covered with xerophytic vegetation. The peninsula is composed of metamorphic rock, forming rocky cliffs. In addition to both flat and inclined sandy bottoms, some coral reef patches are present between 5 m and 25 m depth. Two currents converge at this site. A northern current that provides oceanic waters and a southern current that brings turbid waters with high levels of sediment and nutrients from the Cienaga Grande de Santa Marta (mangrove swamp), Manzanares River and Gaira River.

Playa Cristal (74° 04' W and 11° 19' N) is located in the northeast of Neguange Bay and is part of the Tayrona National Park. It is the biggest bay within the park. Its western side is formed by a rocky littoral zone with a small sandy beach. The area receives freshwater input from the Quebrada Rodriguez River (Little River). Three different types of habitats are found in this area: coral reefs, mangroves and seagrass beds.

Inca-Inca (74° 14' W; 11° 11' N) is located in Gaira Bay, 6 km southeast of Santa Marta. The bay is shallow and has a short shelf that helps bring up deep water to the surface during times of upwelling. The circulation of the currents in this region depends on the circulation of the wind. During the dry period (December through April), a continuous and strong wind from the northeast (Alisios) produces an east-west current running parallel to the coastline. During the rainy period (August through November), a countercurrent running west to east is generated. During that time, a strong wind from the south and southwest (Vendavales) may be present and together with freshwater from the Magdalena River and effluents of the Santa Marta mangrove swamp produce nutrient enriched waters.

To assess abundances, the sampling regime included two 10 x 4 meter quadrants at Inca-Inca and Playa Cristal, and one 10 x 4 meter quadrant at Punta de Betín. However, to augment taxonomic information, additional random searches were conducted. Specimens were collected from under rocks in the littoral zone by gently lifting the animals off the substrate using a small paintbrush. Whole animals were photographed in vivo to record color and color pattern. After photography, animals were coaxed onto pieces of filter paper and placed onto a small amount of frozen 10% buffered formalin. Animals were covered completely with additional fixative and smoothed with a paintbrush to assure that they were fixed flat. This method is a modification of the method of Newman and Cannon (1995). It represents an important improvement over past fixations, which usually had resulted in contorted and broken specimens rendering them useless for taxonomic purposes.

After fixation, morphometric measurements were taken on the worms (e. g., body length and width, ratio of pharynx length to total body length). Paraffin-embedded animals were sectioned sagittally, and the sections were stained with hematoxilin and

eosin. Taxonomic identifications were based on Faubel (1983, 1984a). All material was deposited in the Museo de Historia Natural Marina de Colombia (MHNMC) at the Instituto de Investigaciones Marinas y Costeras (INVEMAR) in Santa Marta, Colombia (Table 1).

Results and Discussion

A total of 372 specimens (25 species in 12 families) were found in the areas surveyed. In terms of absolute abundances, 70.16% was found at Inca-Inca, 18.28% at Punta de Betín, and 11.56% at Playa Cristal (Neguange). Despite having the lowest numerical abundance, Playa Cristal still had the greatest species richness (8 species), followed by Inca-Inca (7 species) and Punta de Betín (3 species). Overall, the most abundant species were *Boninia divae* (76.09%) followed by *Styloplanocera fasciata* (9.16%), *Melloplana ferruginea* (5.57%), Pleioplana sp. (2.42 %), and *Notoplana queruca* (1.88%).

All 25 species found in the rocky littoral of the Santa Marta region represented new records for the Colombian Caribbean. Twenty specimens were identified to species and two (*Notocomplana* sp., *Pleioplana* sp.) were identified to genus (Table 1). An additional specimen was identified to family (Prosthiostomidae), and two specimens have been assigned to the suborder Acotylea. A potentially new species (*Pleioplana* sp.) was found (Table 1) and was the focus of a separate paper (Bolaños et al. 2006). Color and color pattern have been recorded photographically for all but one species (Figure 2).

In his classification system, Faubel (1983) revised the genus *Notoplana* (Notoplanidae) by establishing six new genera (*Chiliplana, Notocomplana, Notoplehnia, Tripyloplana, Pleioplana, Melloplana*) and separating *Melloplana* and *Pleioplana* into a

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newly established Pleioplanidae. He defines the family based on a prostatic vesicle that is completely filled with well-defined, tubular chambers. Species within *Melloplana* are characterized by a papillate penis, whereas those in *Pleioplana* have an armed penis (Faubel 1983). With respect to the Pleioplanidae, we concur with Faubel's (1983) new genera and thus, list *Melloplana ferruginea* in our checklist (table 1).

Taxonomic surveys of polyclads in the Caribbean include Prudhoe (1944), Hyman (1955a, b), and Marcus and Marcus (1968). However, most of the descriptions were based on poorly preserved specimens without color documentation, and often the specimens had been collected by other investigators. Because no color documentation exists for these specimens, it is difficult to recognize actual species using the descriptions of these authors.

Prudhoe (1944) focused on flatworms collected in the Cayman Islands. He described three species of Acotylea and one species of Cotylea. Additional records on 14 acotyleans and 10 cotyleans from the Caribbean were reported by Hyman (1955a, b). These specimens were from the US Virgin Islands, Jamaica, Puerto Rico, Bermuda, the Bahamas, Dominica, and Florida. Most of the specimens were collected by other investigators and were poorly preserved. Again, no color or color patterns were recorded. Marcus and Marcus (1968) described 28 acotyleans and 21 cotyleans from the Lesser Antilles, Puerto Rico, Key Biscayne, and Brazil. Accounting for any overlap among these surveys, a total of 40 acotyleans and 28 cotyleans have been recorded for the Caribbean. Comparing our findings to these previous studies, we find that *Cestoplana rubrocincta, Armatoplana divae, Phaenoplana longipenis, Eurylepta aurantiaca, Thysanozoon* cf. *lagidum*, and *Prosthiostomum gilvum* represent first records for the entire Caribbean region.

In addition, a new combination (*Phrikoceros mopsus* nov. comb.) was recorded. *Phrikoceros mopsus* (Marcus, 1952) had been described previously as *Pseudoceros mopsus* Marcus, 1952. Like *Pseudoceros*, members of *Phrikoceros* have a single male reproductive apparatus, but they are distinguished from that genus by deep marginal ruffling, simple pharyngeal folds and the arrangement of clustered, dorsal and ventral pseudotentacular eyes (Newman and Cannon 1996a). Using these characteristics, the specimens collected in this study clearly belong to the genus *Phrikoceros*, which led us to the establishment of *Phrikoceros mopsus* nov. comb.

Our findings suggest that the Caribbean is indeed a region of high polyclad diversity, although to date, it remains understudied as demonstrated by our new regional records. New methods of fixation (Newman and Cannon 1995) and the addition of photographic records are important and necessary improvements to the study of these animals. Understanding polyclad diversity has implications for many other fields of biology. For example, acotyleans are major predators of sessile marine invertebrates, including commercial bivalves (Pearse and Wharton 1938, Newman et al. 1993, Jennings and Newman 1996a, b). Cotyleans are of importance in the study of aposematic coloration in marine invertebrates (Ang and Newman 1998). Finally, polyclads are of increasing interest in the search for new drugs from the sea (Carté 1996), in fact, it has been shown that some species contain chemicals that can kill human cancer cells (Kubanek et al. 1995). Progress in all these fields though, hinges on a thorough understanding of polyclad systematics, including their distributions and abundances.

Table 1. Polyclads of the Colombian Caribbean. Voucher: vouchers were photographed and deposited at the Instituto de Investigaciones Marinas y Costeras (INVEMAR) in Santa Marta, Colombia as whole mounts; additionally when indicated also as histological sections (HS). Locality: 1: Punta de Betín; 2: Playa Cristal; 3: Inca Inca

Taxon	Voucher	Reference	Locality	Synonyms
ACOTYLEA: DISCOCELIDAE				
Adenoplana obovata (Schmarda,1859)	INV PLA001 INV PLA002 HS	Marcus 1950	3	(a)
ACOTYLEA: CESTOPLANIDAE				
Cestoplana rubrocincta (Grube, 1840)	INV PLA003	Haswell 1907	3	(b)
ACOTYLEA: STYLOCHOPLANIDAE				
Armatoplana divae (Marcus,1947)	INV PLA004	Marcus 1947	3	(c)
Phaenoplana longipenis(Hyman, 1953)	INVPLA007	Hyman 1953	2	(d)
ACOTYLEA: CRYPTOCELIDAE				
Phaenocelis medvedica Marcus, 1952	INV PLA008 INV PLA009 HS	Marcus 1952	2	
ACOTYLEA: NOTOPLANIDAE				
Notoplana queruca Marcus and Marcus 1968	INV PLA0010 INV PLA0011 HS	Marcus and Marcus 1968	2	
Notocomplana sp.	INV PLA 0012 INV PLA0013 HS	Faubel 1983	2	
ACOTYLEA: PLEIOPLANIDAE				
Melloplana ferruginea (Schmarda, 1859)	INV PLA0014 INV PLA0015 INV PLA0016 INV PLA0018 HS	Hyman 1939a, b Hyman 1955a, b Marcus and Marcus 1968	1, 2, 3	(e)
Pleioplana sp.	INV PLA INV PLA0020 HS	Faubel 1983	3	
ACOTYLEA: GNESIOCERIDAE				
Gnesioceros sargassicola (Mertens, 1833)	INV PLA0021 HS	Hyman 1939b, Marcus and Marcus 1968	3	(f)
Styloplanocera fasciata (Schmarda, 1859)	INV PLA0022 INV PLA0023 INV PLA0024 INV PLA0025 HS	Hyman 1955a, Marcus and Marcus 1968	1, 2, 3	(g)
ACOTYLEA:				
Acotylea sp. 1	INV PLA0049	Faubel 1983	3	
Acotylea sp. 2	No voucher deposited	Faubel 1983	2	
COTYLEA: BONINIIDAE				
Boninia divae Marcus and Marcus,1968	INV PLA0026 INV PLA0027 INV PLA0028 HS	Marcus and Marcus 1968	1, 2, 3	
COTYLEA: PERICELIDAE				
Pericelis cata Marcus and Marcus, 1968	INV PLA0030 INV PLA0031 HS	Marcus and Marcus 1968	1, 2	
COTYLEA: PSEUDOCEROTIDAE				
Pseudoceros bicolor Verrill, 1902	INV PLA0032 INV PLA0033 HS	Marcus and Marcus 1968	1, 3	
Phrikoceros mopsus (Marcus, 1952) n. comb.	INV PLA0034 INV PLA0035 HS	Marcus 1952, Marcus and Marcus 1968	3	(h)
Thysanozoon cf. *lagidium* Marcus, 1949	INV PLA0036 INV PLA0037 HS	Marcus 1949	3	

Taxon	Voucher	Reference	Locality	Synonyms
COTYLEA: EURYLEPTIDAE *Eurylepta aurantiaca* Heath and McGregor,1912	INV PLA0038	Hyman 1953	2	
COTYLEA: PROSTHIOSTOMIDAE	INV PLA0050	Faubel 1984	3	
Prosthiostomidae sp. 1	INV PLA0039	Marcus 1950	1, 2, 3	
Prosthiostomum gilvum Marcus, 1950	INV PLA0040 INV PLA0041 INV PLA0042 HS			
Prosthiostomum utarum Marcus, 1952	INV PLA0048	Marcus 1952, Marcus and Marcus 1968	3	(i)
Enchiridium punctatum Hyman, 1953	INV PLA0043 INV PLA0044 HS	Hyman 1953	3	
Euprosthiostomum cf. *adhaerens* Bock, 1925	INV PLA0045	Marcus and Marcus 1968	2	
Euprosthiostomum matarazzoi (Marcus, 1950)	INV PLA0046 INV PLA0047	Marcus 1950, Marcus and Marcus 1968	3	(j)

Synonyms:
(a) *Adenoplana obovata* (Schmarda, 1859) Stummer Traunfels 1933: *Polycelis obovata* Schmarda, 1859; *Leptoplana obovata* (Schmarda) Diesing 1862
(b) *Cestoplana rubrocincta* (Grube, 1840) Lang 1884: *Orthostomum rubrocinctum* Grube, 1840; *Orthostoma rubrocincta* (Grube) Oersted 1844; *Thyphlolepa rubrocincta* (Grube) Stimpson 1857; *Tricelis fasciatus* Quatrefages, 1845; *Cestoplana filiformis* Laidlaw, 1903; *Cetsoplana australis* Haswell, 1907
(c) *Armatoplana divae* (Marcus, 1947): *Stylochoplana divae* Marcus, 1947
(d) *Phaenoplana longipenis* (Hyman, 1953): *Stylochoplana longipenis* Hyman, 1953
(e) *Melloplana ferruginea* (Schmarda, 1859): *Polycelis ferruginea* Schmarda, 1859; *Leptoplana ferruginea* (Schmarda) Diesing 1862; *Discocelis binoculata* Verrill, 1901; *Notoplana bahamensis* Bock 1913; *Notoplana ferruginea* (Schmarda) Stummer Traunfels 1933; *Notoplana binoculata* (Verrill) Hyman 1939; *Notoplana caribbeana* Hyman, 1939
(f) *Gnesioceros sargassicola* (Mertens, 1833): *Planaria sargassicola* Mertens, 1833; *Stylochus sargassicola* (Mertens) Ehrenberg, 1836; *Planocera sargassicola* (Mertens) Oersted 1844; *Stylochus mertensi* Diesing, 1850; *Gnesioceros mertensi* (Diesing) Diesing 1862; *Stylochus pelagicus* Moseley, 1877; *Stylochoplana sargassicola* (Mertens) Graff 1892; *Pelagoplana sargassicola* (Mertens) Bock, 1913
(g) *Styloplanocera fasciata* (Schmarda, 1859) Stummer Traunfels 1933: *Stylochus fasciatus* Schmarda, 1859; *Styloplanocera papillifera* Bock, 1913; *Stylochoplana fasciata* (Schmarda) Lang 1884
(h) *Phrikoceros mopsus* (Marcus, 1952): *Pseudoceros mopsus* Marcus, 1952
(i) *Prosthiostomum utarum* Marcus, 1952: *Lurymare utarum* (Marcus, 1952) Marcus and Marcus 1968
(j) *Euprosthiostomum matarazzoi* (Marcus, 1950): *Prosthiostomum matarazzoi* Marcus, 1950; *Lurymare matarazzoi* (Marcus) Marcus and Marcus 1968

Adenoplana obovata

Armatoplana divae

Phaenocelis medvedica

Notocomplana sp.

Gnesioceros sargassicola

Cestoplana rubrocincta

Phaenoplana longipenis

Enchiridum punctatum

Notoplana queruca

Melloplana ferruginea

Styloplanocera fasciata
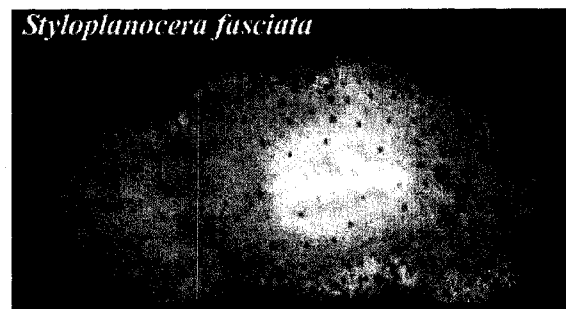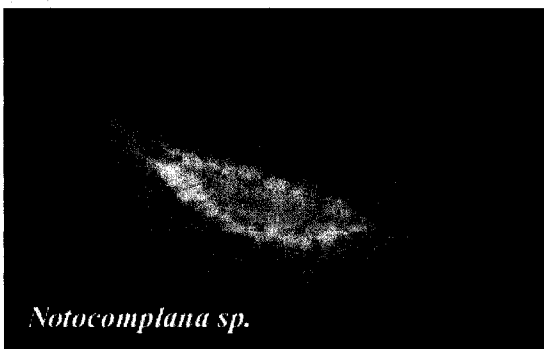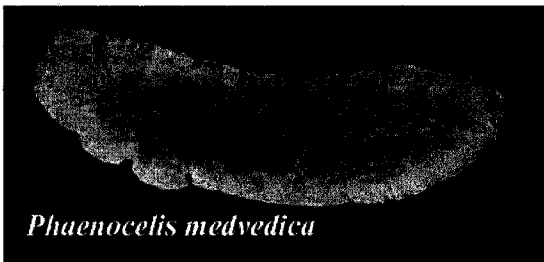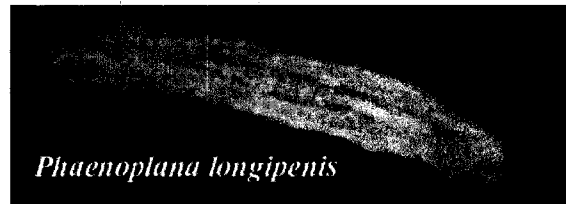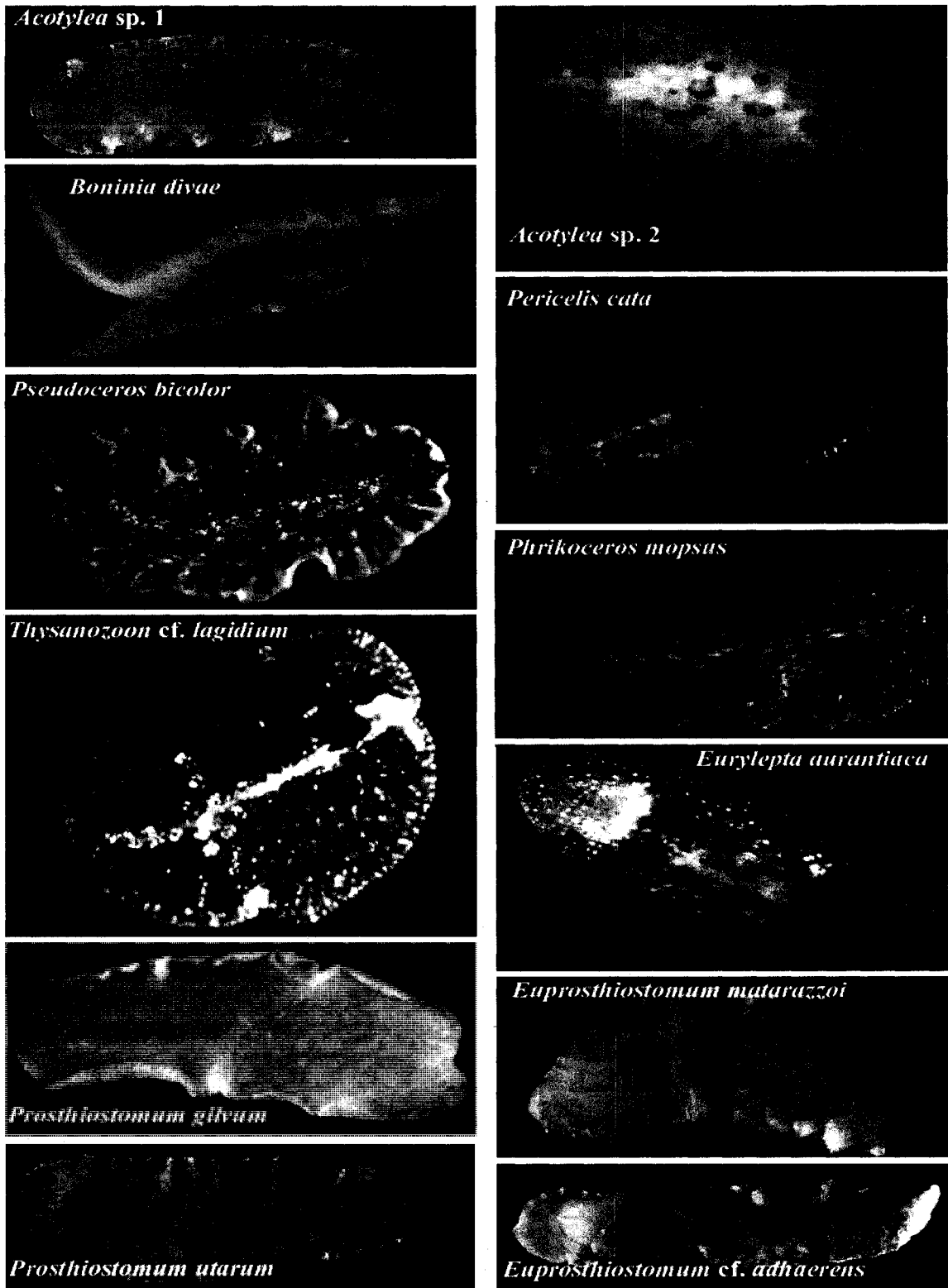
Figure 2. Photographic records of Colombian polyclads

CHAPTER III.

FIRST DESCRIPTION OF DEEP-SEA POLYCLAD FLATWORMS FROM THE NORTH
PACIFIC: *Anocellidus* gen. nov. *profundus* sp. nov. (ANOCELLIDAE, fam. nov.) AND
Oligocladus voightae sp. nov. (EURYLEPTIDAE)[2]

Introduction

Polyclads are free-living, marine flatworms known to inhabit mostly littoral waters.

Their vertical distributions appear limited by temperature, substrate preferences or biotic

associations with seaweeds, corals, sponges, or ascidians (Prudhoe 1985). Typically,

polyclads are benthic, although some pelagic species have been recorded and have

been collected from surface waters (Faubel 1984b); other pelagic species have been

found to depths of almost 1000 m (Palombi 1924). To date, only a single deep-sea

benthic specimen has been recorded, *Stygolepta hjalmari*, from 603 m off the coast of

Mauritania (Faubel 1984a). In fact, according to Herring (2002), free-living flatworms are

not known from the deep sea and any flatworms found at these depths, probably only

occur as parasites of other animals. The paucity of information about deep-sea

flatworms may be due in part to their fragile body constructions, which easily will

disintegrate during traditional trawl collection or to their body shape which allows them to

waft away. Thus, the only way to obtain intact specimens is collecting with submersibles

or Remotely Operated Vehicles (ROVs).

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Here we describe two species of polyclad flatworms collected with such undersea vehicles from wood placed on deep-sea sediments of the Cascadia Basin and Escanaba Trough in the North Pacific Ocean.

Materials and Methods

All specimens were collected by Dr. Janet R. Voight of the Field Museum (FMNH), Chicago Illinois, USA from oak and fir wood blocks that had been deployed in 2002 on the Cascadia Basin and Escanaba Trough in the North Pacific Ocean. Deployments were made at depths of 2642 m and 2660 m at two northern sites, respectively (Baby Bare Seamount at 47° 42.637'N 127° 47.292'W and Ocean Drilling Program (ODP) Drill Hole 1026B at 47° 45.765'N 127° 45.439'W), and to 3232 m depth at Escanaba Trough (41° 0.0272'N 127° 29.679'W). Recovery at the northern sites occurred in July 2003 by the ROV-Jason II (R/V T. G. Thompson) and at Escanaba Trough in August 2004 by the DVS Alvin (R/V Atlantis). Both Cascadia Basin and Escanaba Trough are characterized by heavy sediment associated with turbidite flows from the Pleistocene (Brunner et al. 1999). Details regarding deployment and recovery of the wood blocks and associated fauna can be found in Voight (2005).

Specimens examined here were fixed in 8% buffered formalin in seawater and stored in 70% ethanol. Fixed animals were photographed, and body measurements were taken (measurements given as length mm x width mm). Segments adjacent to and behind the pharynx containing the reproductive structures were removed. Sections were embedded in paraffin, sagittally sectioned at 5-7 μm on an AO 820 (Spencer) microtome, stained with haematoxylin and eosin and mounted in Permount. For whole mounts,

animals were dehydrated, cleared in Histoclear, and mounted in Histomount. Diagrammatic reconstructions of the reproductive system derive from sectioned material and whole mounts. Taxonomic identifications were based on the classification systems of Faubel (1983, 1984a).

Systematics

Suborder: Acotylea Lang, 1884

Superfamily: Ilyplanoidea Faubel, 1984a

Family: Anocellidae fam. nov.

Definition: Ilyplanoidea without eyes. Ruffled pharynx located anteriorly; long, pointed tentacles present. Gonopores separate and male copulatory apparatus positioned posterior to male pore, hence directed forwards; armed with a long and pointed stylet directed backwards. Prostatic-like glands (prostatoid organs, *sensu* Faubel 1983) absent. Spermiducal bulbs present instead of a true seminal vesicle. Female apparatus with Lang's vesicle. A ventral disk of potentially sensory function, positioned anterior to the cerebral ganglion; this organ is not homologous with the cotylean sucker.

Taxonomic remarks regarding the new family: According to Faubel (1983), the absence of a true prostatic vesicle is the taxonomic character defining the superfamily Ilyplanoidea which includes the families Enantiidae Graff 1889, Discocelidae Laidlaw 1903, Discoprosthididae, Euplanidae, Ilyplanidae, Mucroplanidae and Paluidae (the last five all by Faubel 1983). The ruffled pharynx and a male tract without a trace of prostatic-like glands or a prostatic vesicle would place this new species into the genus

Aprostatum, within the Euplanidae. This family is defined by a ruffled, centrally located pharynx, a posteriorly-dircted male copulatory apparatus, separate gonopores, and a complete lack of prostatic-like glands (Faubel 1983). Although these characters conform to those found in our new species, an additional character found in our specimens warrants the establishment of the new family Anocellidae.

The defining character of the Anocellidae is that the male copulatory apparatus is located posterior to the male gonopore and is directed anteriorly. This is exactly opposite of the condition found in the Euplanidae, where the male copulatory complex is located anterior to the male gonopore and directed posteriorly (Table 2). Using a single character as a basis for a new family without a complete reanalysis of acotylean classification may appear precipitous, however, "orientation of male copulatory apparatus" weighs heavily in the classification system of acotyleans and has previously been used in defining families (Faubel 1983). In fact, Lang (1884) used it as the main character to establish the Cestoplanidae, a group in which the male copulatory apparatus is located posterior to the male gonopore and is directed anteriorly. Other morphological differences however eliminate placement of the new species in the Cestoplanidae (Table 2), thus justifying the establishment of the family Anocellidae.

Key for the determination of the new family (modified from Faubel 1983)

1. Ruffled pharynx..2

   - tubular or cylindrical pharynx, directed forwards...................**Enantidae**

2. male tract provided with prostatic like glands......................................3

   - male tract completely lacking prostatic like glands.............................7

3. male tract with glandular epithelium, prostatoid organs lacking..............6

   - male tract with or without glandular epithelium, prostatoid organs present...4

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4. male tract without prostatic-like lining..5

    - male tract with prostatic-like lining.......................**Discoprosthididae**

5. male tract edged with numerous prostatoid organs.............**Discocelidae**

    - male tract without prostatoid organs; a single armed prostatoid organ opens

    independently from the male tract to the

    exterior..**Palauidae**

6. like (3) and with bulbous, glandular chambered penis, prostatic-like glands are

    extraepithelial...**Mucroplanidae**

    - penis if present, is papilla-like rod-like or armed with a cuticular

    stylet...**Ilyplanidae**

7. male copulatory apparatus positioned anterior to male pore, hence directed

    backwards...**Euplanidae**

    - male copulatory apparatus positioned posterior to the male gonopore, hence

    directed forwards; eyes lacking ....**Anocellidae**

Table 2. Comparison of morphological features of the new family Anocellidae with Euplanidae and Cestoplanidae.

Character	Anocellidae	Euplanidae	Cestoplanidae
Body form	Round to oval	Elongate, oval or cuneate	Very elongate, slender
Tentacles	Nuchal tentacles present	Absent or rudimentary	Absent
Eyes	Absent	Well-developed marginal eyes; tentacular, frontal and cerebral eyes-spots present	Eye-spots scattered over anterior end; definite cerebral and tentacular eyes absent
Prostatic vesicle	Absent	Absent	Present, interpolated
Location of male copulatory complex	Posterior to male gonopore	Anterior to male gonopore	Posterior to male gonopore
Orientation of male copulatory complex	Directed anteriorly	Directed posteriorly	Directed anteriorly
Ventral, sensory disk	Present	Absent	Absent

Genus: _Anocellidus_ gen. nov.

Definition: with the characters of the family

Species: _profundus_ sp. nov. (Figs. 3-7)

Type Material and Locality:

a) Holotype, whole mount, one mature specimen (10 mm X 10 mm), FMNH 12555; collected 11 July, 2003 from 2660 m depth at ODP 1026B (47°45.765'N 127°45.439'W).

b) Paratype, one mature specimen as serial sagittal sections (13 mm X 12 mm), FMNH 12556; collected with holotype on 11 July, 2003 from 2660 m depth at ODP 1026B (47°45.765'N 127°45.439'W).

Other Material Examined:

c) Whole mount, one mature specimens (11 mm X 10 mm), FMNH 12557; collected with holotype on 11 July, 2003 from 2660 m depth at ODP 1026B (47°45.765'N 127°45.439'W).

d) One mature specimen, as serial sagittal sections (11 X 10 mm), FMNH 12558; collected with holotype on 11 July, 2003 from 2660 m depth at ODP 1026B (47°45.765'N 127°45.439'W).

e) One mature specimen, as serial sagittal sections (11 mm X 10 mm), FMNH 12559; collected 3 July, 2003 from 2642 m depth at Baby Bare Seamount (47° 42.637'N 127° 47.292'W).

f) Ethanol-preserved specimen, not sectioned or mounted, FMNH 11736; collected with holotype

g) Ethanol-preserved specimen, not sectioned or mounted, FMNH 11778; from Baby Bare Seamount.

h) Ethanol- preserved specimen, not sectioned or mounted, FMNH 12463; collected on 30 Aug, 2004 from Escanaba Trough, 20 m N of Marker 6X on Central Hill, from 3232 m depth (41° 00.272'N 127° 29.679'W).

Etymology: name from an = without, ocell- = little eye, for the absence of eyes, and profundus = deep, a reference to the depth at which the specimens were collected.

Distribution: To date, known from the type locality ODP 1026B (47°45.765'N 127°45.439'W), from Baby Bare Seamount (47° 42.637'N 127° 47.292'W), and from Escanaba Trough (41° 00.272'N 127° 29.679'W) at depths of from 2642 to 3232 m.

Diagnosis: Male copulatory apparatus posterior to the male pore and directed anteriorly, with a long and pointed stylet directed posterior; prostatic vesicle and prostatic-like glands absent. Well-developed, nuchal tentacles, eyes completely lacking. Ventral sensory disk present anterior to the cerebral ganglion.

Description

External features: *Color*-- Live and preserved specimens are whitish but testes and ovaries appear as numerous brown dots visible through the epidermis (Fig. 3). In cleared specimens, testes and ovaries arrayed radially about the pharynx; testes appear smaller and dark yellow.

Form-- Preserved specimens range from 10 mm x 10 mm to 13 mm x 12 mm and have a fleshy, rounded body (Fig. 3A). The body margin is smooth without evident folds. Ventrally, an arrowhead-shaped organ of putative sensory function lies just anterior to the cerebral ganglion (Figs. 3B, 4, and 5).

Tentacles-- long, pointed, non-retractile nuchal tentacles are present (Fig.3A).

Eyes-- absent.

Digestive system-- a ruffled and very muscular pharynx with 4-5 deep folds on both sides is medial in the anterior third of the body (Figs. 3B, 4 and 6). A small mouth is present in the anterior part of the pharynx. In histological sections, a medial intestinal branch extends anteriorly dorsal to the brain and posteriorly dorsal to Lang's vesicle (Fig 5B). Three to four radial intestinal branches are very conspicuous on either side of the pharynx.

Reproductive anatomy: *Gonopores--* male gonopore located just posterior to the mouth and ventral to the pharynx; female gonopore positioned medially, immediately posterior to the male spermiducal bulbs.

Male copulatory apparatus-- located posterior to the male pore and directed anteriorly; the stylet, however, is recurved (Fig. 7). The prostatic vesicle is lacking and there is no evidence of a prostatoid organ or prostatic-like glands around the ejaculatory duct or of any glandular epithelium in the male tact. A seminal vesicle and vasa deferentia are absent but two highly muscularized spermiducal bulbs are present lateral and slightly posterior to the pharynx. The spermiducal bulbs appear as a W-shape in whole mounts (Fig. 4) and they fuse to form a single ejaculatory duct. The sinuous ejaculatory duct continues into a very long, thin and pointed stylet (Figs. 3B, 6, and 7). A very thin, deep

stylet pocket parallels the ventral surface of the worm just ventral to the ejaculatory duct (Fig. 7). The male atrium is shallow and the stylet was found extruded in most specimens (Figs. 3B and 7).

Female apparatus--shallow female atrium. The vagina is spacious and curves posterior, terminating in a large, elongated Lang's vesicle (Figs. 4, 5B, 6 and 7). The uteri lie on either side of Lang's vesicle and the oviducts form a loop before joining with the vagina immediately proximal to Lang's vesicle (Fig. 6).

Reference measurements from the largest *(13 mm x 12 mm) available specimen*

brain, 375 µm in diameter; Lang's vesicle, 1.75 mm long; pharynx, 3mm long; stylet, 1037 µm X 75 µm; spermiducal bulbs, 300 µm maximum diameter; tentacles, 625 µm long X 250 µm maximum diameter at their base.

Taxonomic Remarks

Although the classification system of Prudhoe (1985) is more conservative because it recognizes fewer taxa, the use of Faubel (1983, 1984a) is appropriate because of its importance on characters of the reproductive system. *Anocellidus profundus* lacks a prostatic vesicle and in the system of Faubel (1983, 1984a), would be placed in the Ilyplanoidea. This superfamily corresponds to the Cestoplanoidea of Prudhoe (1985). Prudhoe (1985) subdivided the Acotylea into three superfamilies depending on the position of eyes, recognizing marginal, tentacular, cerebral, and frontal eyes. Absolute eye positions are often difficult to determine with areas of cerebral, marginal, and frontal eyes overlapping. And although in his lower taxonomic units, Faubel (1983, 1984a) does place importance on what we consider minor or

developmentally plastic details (e. g., type of lining of the prostatic vesicle), the presence

or absence of an entire structure such as the prostatic vesicle, does appear to be a

much more reliable character than the position of eyes.
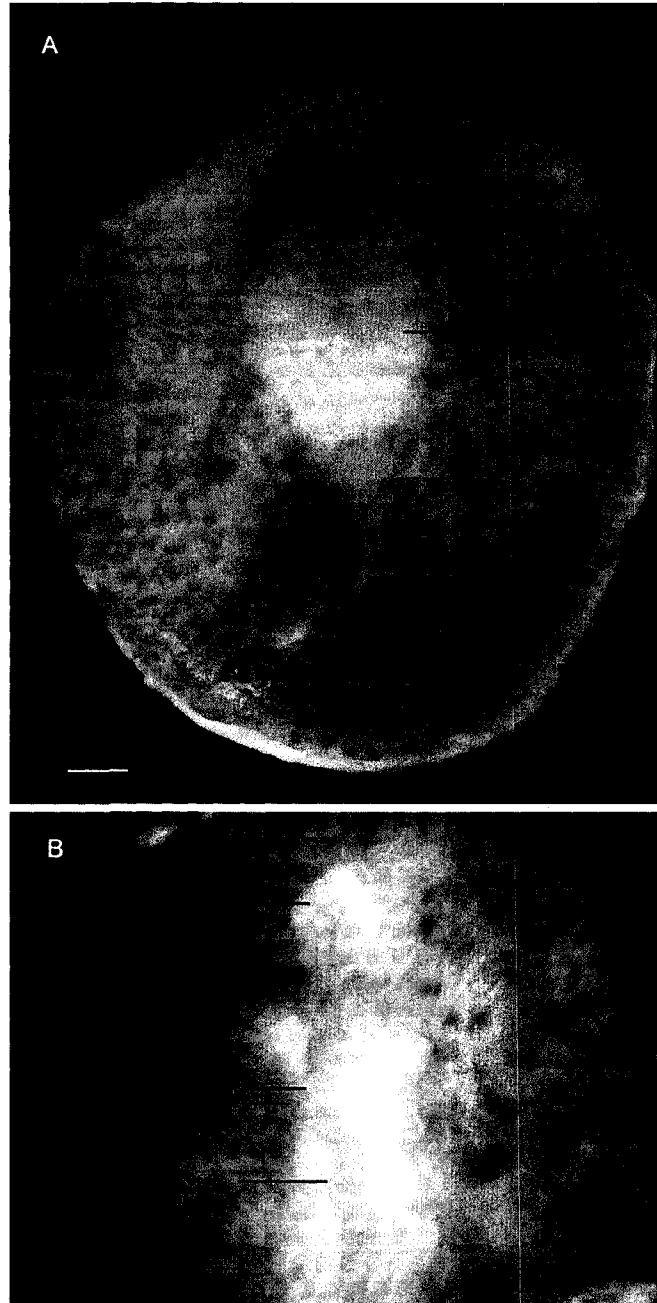
Figure 3. Preserved specimen of *Anocellidus profundus* sp. nov.; photomicrographs. A. Anterior end, showing nuchal tentacles, highly folded pharynx, and uteri. Scale bar = 1 mm. B. Higher magnification of sensory organ. Note extruded stylet. Scale bar = 1 mm.
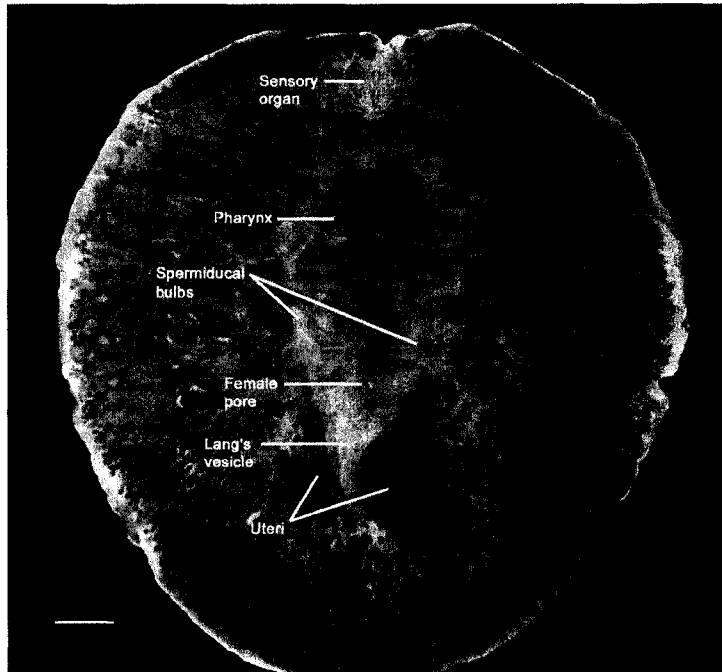
Figure 4. Cleared whole mount of *Anocellidus profundus* sp. nov., showing sensory organ, pharynx, and structures of the male and female reproductive complexes. Scale bar = 1 mm.
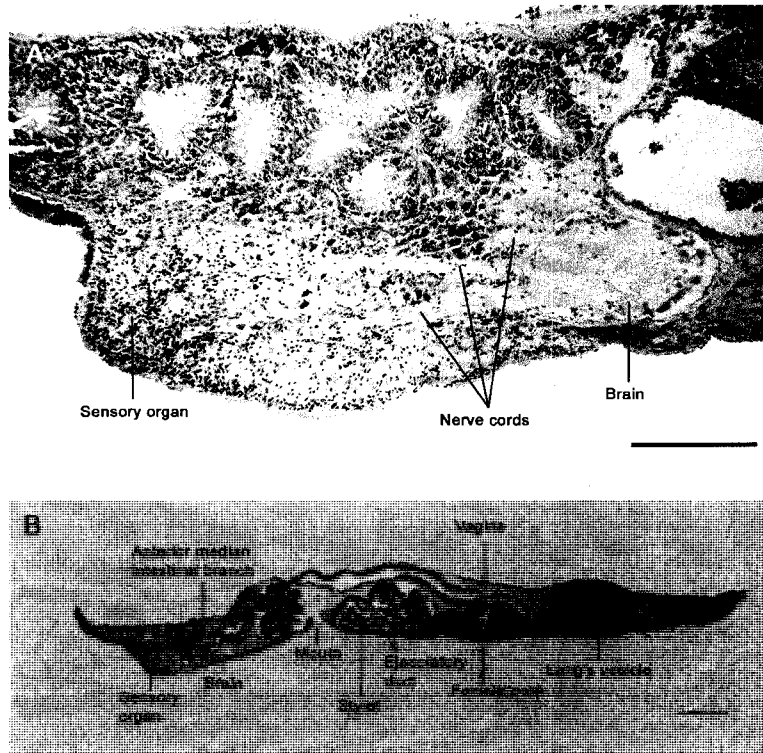
Figure 5. *Anocellidus profundus* sp. nov. photomicrographs. A. Sagittal histological section through the anterior end. Nerve cords can be seen extending from the brain to the sensory organ. Scale bar = 250 μm. B. Sagittal histological section, showing reproductive and digestive structures. Scale bar = 1 mm.

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Even though the reproductive anatomy of *Anocellidus profundus* is somewhat similar to that of species in the euplanid genus *Aprostatum*, it clearly does not pertain to that genus (Table 3). The presence of nuchal tentacles, the total absence of eyes, and the presence of a unique ventral sensory organ also separate *A. profundus* from all other members of the Euplanidae (Table 2). In fact, current classification interprets the defining character of "orientation of male reproductive complex" in *A. profundus* as meriting familial status.

Here we note the presence of tentacles in *Anocellidus profundus*, although the character's systematic value is uncertain. For example, Faubel (1983) defines the genus *Armatoplana* as lacking tentacles, however, *Armatoplana divae* has tentacles (Faubel 1983), as does *A. colombiana* (Bolanos et al., 2006). Also the absence of eyes in *A. profundus* may relate more to its environment than to its phylogenetic history.

However, we recognize the ventrally located putative sensory organ anterior to the cerebral ganglion as an unusual character. A cursory examination of this disk may result in its being confused with a cotylean sucker and the mistaken placement of the specimens in the Cotylea. The presence or absence of a true sucker posterior to the female gonopore has defined the suborders Cotylea (with sucker) and Acotylea (without sucker) (Lang, 1884). However, exceptions exist; species of acotyleans with a sucker include *Leptoplana tremellaris* (O.F. Müller 1774) and *Itannia ornata* Marcus 1947, although these suckers are never positioned behind the female gonopore (the typical sucker position in cotyleans). In *L. tremellaris*, the sucker is a depression between the male and female gonopore along the midline of the body (Faubel 1983). In *I. ornata*, two adhesive organs are posterior to the gonopore on either side of the midline (Marcus 1952). Despite the presence of such suckers, other characteristics, such as position of the tentacles, structure of the copulatory complex, and arrangement of eyes determine

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the placement of *I. ornata* into the Acotylea (Bock 1913, Hyman 1951, Faubel 1983,

1984a, Prudhoe 1985). Similarly, the presence of a Lang's vesicle and nuchal tentacles

in *Anocellidus profundus* clearly places this species in the Acotylea.
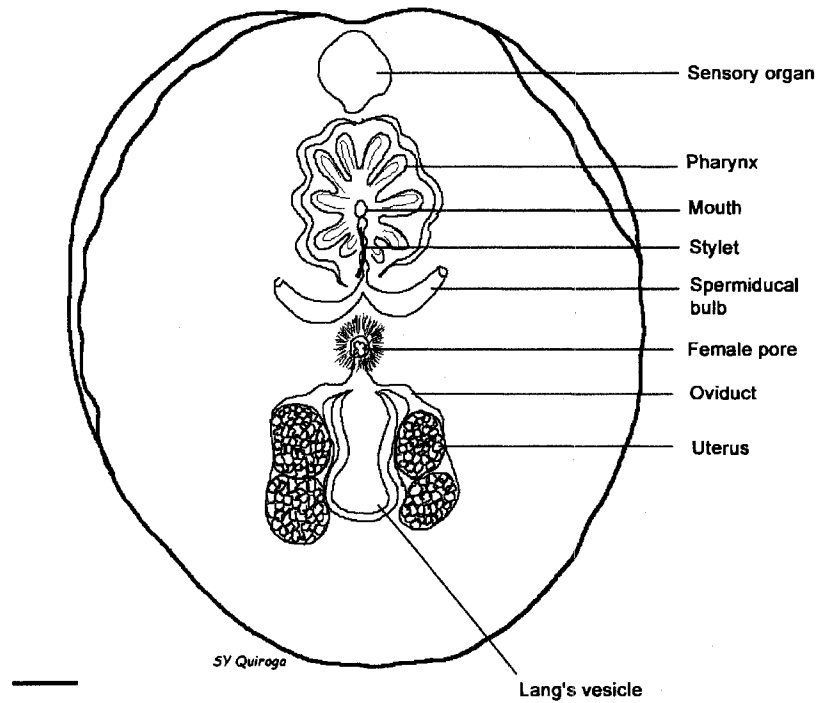
Figure 6. Diagram of ventral view of *Anocellidus profundus* sp. nov. Scale bar = 1 mm.
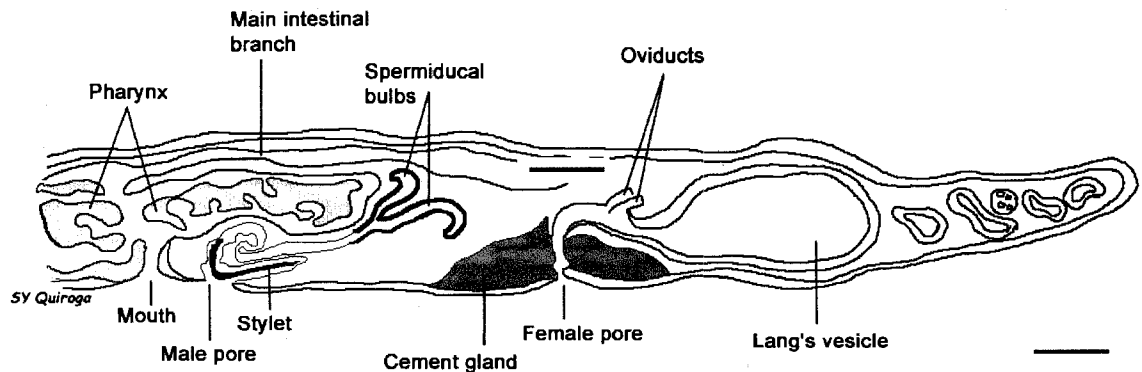
Figure 7. Schematic representation of the male and female reproductive structures of *Anocellidus profundus* sp. nov. Scale bar = 250 μm.

Three noteworthy characteristics of this putative sensory organ of *Anocellidus profundus* show that it is not homologous with the cotylean sucker. First, lateral nerve cords appear to innervate the organ (Fig. 5A), secondly, rhabdites are completely lacking from the organ's epithelium (a distinct characteristic of cotylean suckers), and finally, the organ is located just subterminal to the anterior margin. From these observations, it appears then that this organ may serve a sensory rather than an adhesive function. However, additional studies are certainly needed to confirm this.

Table 3. Comparison of morphological features of species in the genus *Aprostatum** (i. e., lacking a prostatic vesicle and/or prostatoid organs) and the new species *Anocellidus profundus*

Species	Pharynx	Eyes	Tentacles
Anocellidus profundus	Anterior with 4-5 folds	Absent	Long, pointed nuchal tentacles
*Aprostatum stiliferum**	Numerous folds	Marginal eyes in irregular rows surrounding entire body; numerous small eyes in fan-shape over cephalic region	Absent
Aprostatum clippertoni	Small, with small lateral folds	As in *A. stiliferum*	Absent
Aprostatum longipenis	Morphologically uniform	Present	Absent

Species	Ventral Sensory Organ	Spermiducal Bulbs	Seminal Vesicle	Penis Stylet	Lang's Vesicle
Anocellidus profundus	Anterior to cerebral ganglion	Present without vasa deferentia	Absent	Long pointed directed backwards	Large, oval
*Aprostatum stiliferum**	Absent	Present	Absent	Tubular, pointed	Large
Aprostatum clippertoni	Absent	Absent	Absent	Thin, short	Small
Aprostatum longipenis	Absent	Present	Absent	Tubular, pointed	Large

*The status of the genus *Aprostatum* needs re-evaluation. Bock (1913) designated *Aprostatum stiliferum* as the type species of that genus, listing its lack of either a prostatoid organ or a seminal vesicle as a defining character. However, an examination of material from Chile showed the presence of both a seminal vesicle and a prostatic organ in *A. stiliferum* (Marcus 1954).

Suborder: Cotylea Lang, 1884

Superfamily: Euryleptoidea

Family: Euryleptidae

Genus: *Oligocladus*

Species: *voightae* sp. nov. (Figs. 8-12)

Type Material and Locality:

a) Holotype, whole mount, one mature specimen (12 mm x 10 mm), FMNH 12560, collected 30 August, 2004 from Escanaba Trough, 20 m N of Marker 6X on Central Hill, from 3232 m depth (41° 00.272'N 127° 29.679'W).

b) Paratype, one mature specimen as serial sagittal sections (11 mm x 9 mm), FMNH 12464, collected with holotype 30 August, 2004 from Escanaba Trough, 20 m N of Marker 6X on Central Hill, from 3232 m (depth 41° 00.272'N 127° 29.679'W).

Distribution: To date, found only at type locality

Etymology: Species named in honor of Dr. Janet Voight of the Field Museum of Natural History, Chicago, Illinois.

Diagnosis: Mouth anterior to the brain. Eyes, few and minute, scattered on the tentacles. Seminal vesicle connected posteriorly to an auxiliary storage vesicle containing a basophilic substance (possibly sperm). Auxilliary storage vesicle extends dorsally over the seminal vesicle. Posterior anal pore in main median branch of the intestine.

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Description

External features: *Color*-- preserved animals have a milky white dorsal surface with ovaries appearing as dark brown spots that form a radial pattern. White intestinal branches are visible through the epidermis. The ventral surface is white.

Form-- oval body shape, margins without folds except for the ones forming the tentacles. The two specimens measured 12mm x10 mm and 11 mm x 9 mm, respectively. A very conspicuous sucker is located right in the center of the ventral surface, posterior to the pharynx (Figs. 11 and 12).

Tentacles-- Short (~600 μm), blunt, marginal tentacles formed mainly by the elongation of the body margin rather than the folding of it (Fig. 8).

Eyes-- Few and minute, scattered over the tentacles. Cerebral and marginal eyes absent.

Digestive system-- the mouth is a small opening, anterior to the brain (Fig. 10A). The very muscular and cylindrical pharynx is directed forward and located in the anterior half of the worm, just anterior to the sucker. It folds back on itself in an S-shape, possibly due to a preservation artifact (Figs. 10A and 12). The pharynx connects to a median intestinal branch which itself extends anteriorly dorsal to the brain, and posteriorly almost to the body margin. The posterior part of the median intestinal branch divides into 6 to 8 large, radial branches and 4 smaller ones just dorsal to the pharynx (Fig. 9). An anal pore is present on the medial intestinal branch just prior to the terminal end of the branch itself (Fig. 9). An anterior vesicle opening to the exterior was observed, but there is no evidence of it being connected or related to the digestive system.

Reproductive anatomy: *Gonopores*-- pores are well separated from each other. The female pore is posterior to the male pore and located well anterior of the sucker.

Male copulatory apparatus-- testes are scattered ventrally all over the body but are especially concentrated in the posterior end. The male apparatus is rather small compared to the size of the animal. A free prostatic vesicle (125 μm) is located just dorsal to the male atrium (Fig. 10). The seminal vesicle is larger than the prostatic vesicle (175μm), and is located posterior to the atrium. In addition, the seminal vesicle is connected to an accessory storage vesicle containing a basophilic substance, possibly sperm (Figs. 10 and 12). This accessory vesicle wraps itself dorsally around the seminal vesicle. A deep atrium houses a pointed stylet (175μm).

Female copulatory apparatus-- The ovaries are dorsal and scattered over the entire body, fanning out in a radial pattern from the pharynx (Fig. 8A). Two small uterine trunks are present behind the pharynx. Numerous uterine vesicles are present (Figs. 8A and 11). The female atrium is not very deep and connected to a simple vagina, which in turn connects to oviducts.

Taxonomic Remarks:

Both, Faubel (1984a) and Prudhoe (1985) are in agreement of the genus *Oligocladus* in the Euryleptidae. Therefore, either classification system may be used for its identification. Conspicuous characters such as a digestive system with a median main intestinal branch, a plicate, cylindrical pharynx extending anteriorly to the level of the brain, a male copulatory complex located anterior to the female one, and a true free prostatic vesicle, clearly place this species in the family Euryleptidae. Among the

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Euryleptidae, three genera share the possession of an anal pore in the main intestinal branch, *Oligocladus, Cycloporus*, and *Leptoteredra*. However, only *Oligocladus* and *Cycloporus* also possess a pair of uterine trunks with multiple uterine vesicles (Faubel 1984a).
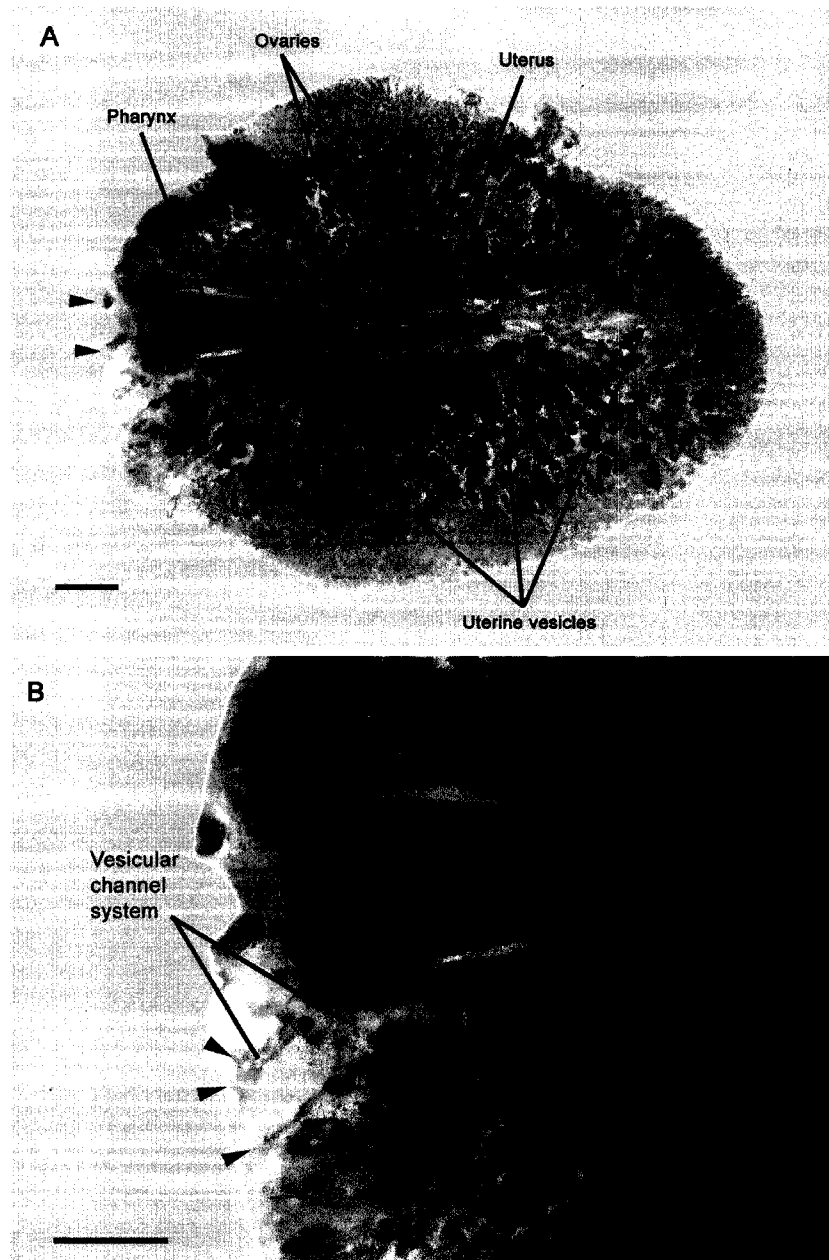
Figure 8. Whole mount of *Oligocladus voightae* sp. nov.; photomicrographs. A. Anterior end, showing tentacles (arrow heads), massive pharynx, and female reproductive structures. Scale bar = 1 mm. B. Higher magnification of anterior end. Arrow heads indicate putative pores of vesicular channel system. Note, channels are only found on one side of the animal. Scale bar = 1 mm.
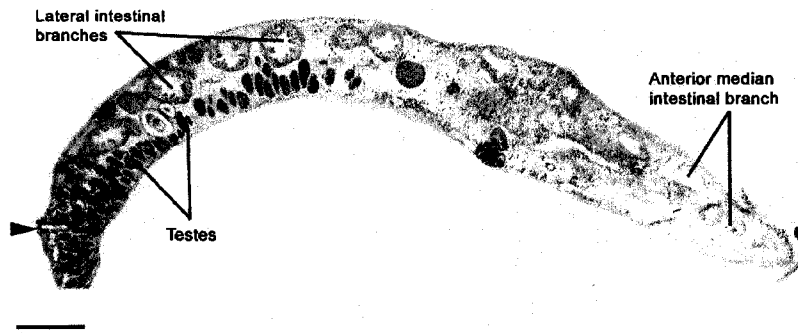
Figure 9. Sagittal section of *Oligocladus voightae* sp. nov., showing details of intestine and characteristic anal pore (arrow). Scale bar = 1 mm.
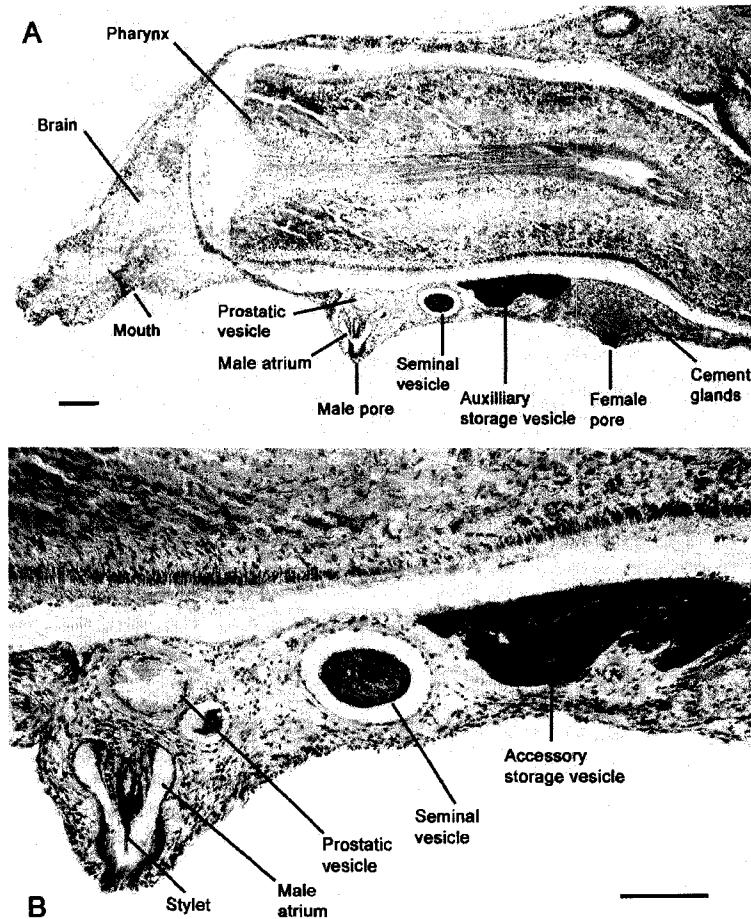
Figure 10. *Oligocladus voightae* sp. nov.; photomicrographs. A. Sagittal histological section of the anterior end showing position of brain posterior to mouth and details of the male and female reproductive systems. Scale bar = 150 μm. B. Higher magnification sagittal section through male copulatory complex. Asterisk indicates the connecting duct between the seminal vesicle and the accessory storage vesicle. Scale bar = 100 μm.
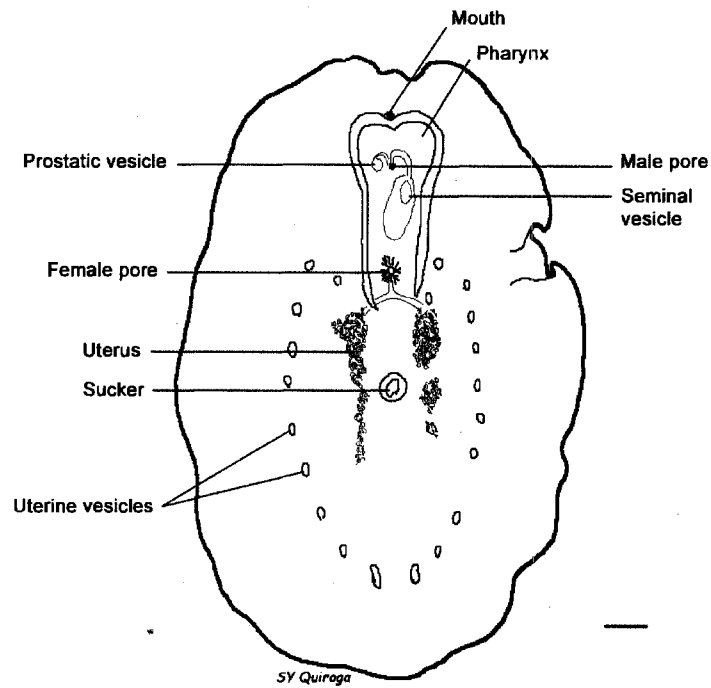
Figure 11. Diagram of ventral view of *Oligocladus voightae* sp. nov., showing position of male and female reproductive structures. Scale bar = 1 mm.
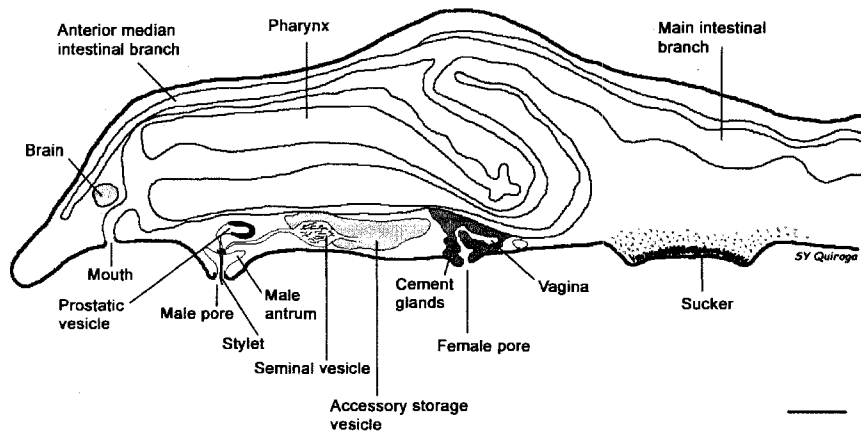
Figure 12. Schematic sagittal representation of reproductive and digestive systems of *Oligocladus voightae* sp. nov. Scale bar = 250 μm.

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Oligocladus and *Cycloporus* are distinguished by the presence of many peripheral vesicles opening to the exterior. In *Cycloporus* these vesicles have clear connections to the digestive system; in *Oligocladus* they do not. In one examined specimen, a vesicular channel system opening via several pores to the exterior was observed in the anterior portion, albeit only on one side of the worm. However, the relationship of this channel system to the digestive system is uncertain. In addition, an obvious anal pore opening dorsally from the caudal end of the main intestinal branch confirmed a placement into *Oligocladus* rather than *Cycloporus.*

Additionally, Hadenfeldt (1929) recognizes the position of the brain posterior to the mouth as unique to the genus Oligocladus, with only two species. According to Faubel (1984a), the anatomy of *O. auritus* is not very well known, although Lang (1884) placed it into this genus because its brain is posterior to the mouth. Thus, the position of the brain of *O. voightae* supports the placement of this species in the genus *Oligocladus.* Furthermore, the general anatomy of *O. voightae* corresponds well with that of *O. sanginolentus* with the exception of the presence of two sharply-defined clusters of cerebral eyes and an anteriorly trifurcated intestinal trunk in the latter species (Prudhoe, 1985).

Ecological Remarks:

It is interesting to note that the two newly described species so far have been collected only from deep-sea wood deployments heavily colonized by wood-boring clams (species of which are currently being described, Dr. J. Voight, Chicago Field Museum, pers. comm.). Previously, Turner (1978) demonstrated the importance of deep-

sea wood deployments in the establishment of a highly diverse community, consisting of wood-boring bivalves, predatory polychaetes and gastropods, galatheid crabs, echinoderms and most likely fish. With respect to flatworms, Turner (1978) reports a single specimen of a predatory turbellarian only; the most abundant taxa of her study being gastropods and polychaetes.

This contrasts sharply with polyclad densities found at Escanaba Trough and ODP. In August 2004, 149 specimens were collected from four 18 inch x 4 inch x 4 inch pieces of lumber at Escanaba Trough that had been deployed there in July 2002, and in July 2003, 47 specimens were collected from ODP 1026B and 33 specimens from near Baby Bare Seamount off identical deployments made in September 2002 (Dr. J. Voight, pers. comm.).

The ecological function of the two newly described species may be inferred from known predatory behavior of other polyclads. Acotyleans of the families Leptoplanidae and Stylochidae are known to prey on rock and pearl oysters (Newman et al. 1993, O'Connor and Newman 2001), blue mussels (Galleni et al. 1980, Villalba et al. 1997), barnacles (Murina et al. 1995), and cultured giant clams (Newman et al. 1993). Their impact can be substantial, as they often feed exclusively on spat or juvenile bivalves, resulting in as much as 90% mortality (Newman et al. 1993). Predation by a stylochid polyclad on oysters has been reported also for the US Atlantic and Gulf of Mexico coasts (Provenzano 1961, Webster and Medford 1961, Christensen 1973, Chintala & Kennedy 1993), and Newell et al. (2000) were able to show that small (< 5 mm2) polyclads were instrumental in significantly reducing the numbers of young oyster spat (less than 3 weeks post-settlement) in Chesapeake Bay.

Recently, Ritson-Williams et al. (2006) described the predatory behavior of a planocerid, concluding that this flatworm uses tetrodotoxin to capture and kill its mollusk prey. From the observed ecological association of *Anocellidus profundus* and *Oligocladus voightae* with deep-sea bivalves, it is tempting to speculate that the flatworms may use a toxin such as tetrodotoxin to subdue their prey. At this point, however, such predator-prey interactions, and the presence of toxins in deep-sea flatworms remain to be demonstrated.

CHAPTER IV.

TWO NEW SPECIES OF FLATWORMS (PLATYHELMINTHES: POLYCLADIDA)
FROM THE CONTINENTAL SLOPE OF THE GULF OF MEXICO[3]

Introduction

The Gulf of Mexico is a marginal basin of the Atlantic Ocean and possesses a

high number of unusual bathyal habitats ranging in size from large hydrocarbon seeps to

relatively small wood falls. Cold hydrocarbon seeps often co-occur with hypersaline

brines caused by exposure of the Louann salt layer (Salvador 1987; MacDonald et al.

1990). Some brine flows fill bottom depressions to form pools, which are characterized

by distinct microbial and macrofaunal assemblages that resemble the fauna of

hydrothermal vents (Paull et al. 1984; Kennicutt et al. 1985, 1988; Cordes et al. 2007).

The cold seep known as Brine Pool NR-1, is filled with brine containing large amounts of

methane gas and is completely surrounded by dense beds of *Bathymodiolus childressi*

(MacDonald et al. 1990). The mussels harbor methanotrophic symbionts (Childress et al.

1986; Cary et al. 1988) and have been shown by stable isotope analyses to be a food

source for vertebrate and invertebrate predators (MacAvoy et al. 2003). Other taxa

associated with Brine Pool NR-1 include chemosynthetic tube worms, several species of

crustaceans, and demersal fish (MacDonald et al. 1990).

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[3] Quiroga S, Bolaños M, Litvaitis MK. 2008. Two new species of flatworms (Platyhelminthes: Polycladida) from the continental slope of the Gulf of Mexico. Journal of the Marine Biological Association of the United Kingdom (in press).

Wood falls provide periodic pulses of organic matter to the deep sea, and can sustain distinct communities that resemble assemblages associated with whale falls, hydrothermal vents, and cold seeps (Van Dover 2000). The Mississippi River serves as a ready source of wood in the northern Gulf of Mexico. Trophic and ecological relationships among animals on wood falls have been described and include distinct opportunistic wood-borers, fouling organisms, and predators (Turner 1978; Pailleret et al. 2007). To date, the only flatworms reported from wood falls are an unidentified predatory turbellarian (Turner 1978) and two recently described species of polyclads, *Anocellidus profundus* and *Oligocladus voightae* from the Cascadia Basin and the Escanaba Trough in the North Pacific Ocean at depths of 2639 to 3232 m (Quiroga et al. 2006). Lastly, in Van Dover et al. (2001), Fig. 2E shows deep-sea flatworms at hydrothermal vents of the Indian Ocean, and although no positive identifications are provided, the flatworm specimens are, in all likelihood, polyclads.

Similarly, the polyclad fauna from bathyal environments (200-2000 m) still remains largely unknown. Faubel (1984a) mentions *Stygolepta hjalmari* from 603 m depth off the coast of Mauritania. Other polyclads from similar depths include *Plehnia arctica*, collected from 1275 m at Jan Mayen Island in the Arctic Ocean (Bock 1913) and *Discoprosthides patagoniensis*, reported from the bathyal zone (no specific depth given) off Argentina (Faubel 1983). *Stylochus crassus* has been found in samples collected at 2000 m deep off the coast of New England, USA; however, the species is presumed to live in association with floating algae rather than on the sea floor (Verrill 1892). Here we add two new species collected from a hypersaline cold seep and from a natural wood fall in the northern Gulf of Mexico to the known polyclad fauna of bathyal environments.

Materials and Methods

All specimens were collected by Dr. Janet R. Voight of the Field Museum (FMNH), Chicago Illinois, USA. Once located, the wood fall was picked up with the manipulator arm of the crewed submersible, Johnson SeaLink, and placed in a lidded box for recovery. The sample from Brine Pool NR-1 was collected among mussels with a scoop integral to the Johnson SeaLink and transferred to plexiglass containers for recovery. Specimens examined here were fixed in 8% formalin in seawater and are stored in 70% ethanol. Fixed animals were photographed, and body measurements were taken (measurements given as length mm x width mm). Animals were processed following the protocol of Quiroga et al. (2006), and diagrammatic reconstructions of the reproductive system were derived from sectioned material and whole mounts. Taxonomic identifications follow the classification of Faubel (1983, 1984a).

Systematics

Suborder: Acotylea Lang, 1884

Superfamily: Leptoplanoidea Faubel, 1984a

Family: Didangiidae Faubel, 1983

Genus: *Didangia* Faubel, 1983

Species: *carneyi* sp. nov. (Fig. 13-16)

Type Material and Locality:

a) Holotype: one mature specimen (9mm X 6mm) as sagittal sections of reproductive structures, remainder of specimen as whole mount (FMNH 13968, 5 slides) (USA: Louisiana, Gulf of Mexico from a wild wood fall at Station VOIJ-JSLII-3531, JSL Dive number 3531; coordinates: 27°44.09'N 91°14.49'W, water depth: 610 m). Collected on 19 August 2006.

b) Paratype: one mature specimen (11mm X 5mm) as sagittal sections of the reproductive structures, remainder of specimen as whole mount (FMNH 13969, 4 slides). Collected with the holotype.

Other Material: (all collected with the holotype)

a) One mature specimen (7mm X 3.5mm), as sagittal sections of the reproductive structures, remainder of specimen as whole mount (FMNH 13775E, 3 slides).

b) Two juveniles (4mm X 2.5mm; 6mm X 3mm), as whole mounts (FMNH 13775F, 2 slides).

c) One mature specimen (5mm X 3mm), as sagittal sections of the reproductive structures (FMNH 13775A, 4 slides).

d) One mature specimen (8mm X 5mm), as whole mount (FMNH 13775C, 1 slide).

e) One mature specimen (10mm X 7mm), as sagittal sections of the reproductive structures, remainder of specimen as whole mount (FMNH 13774C, 6 slides).

f) One mature specimen (7mm X 6mm) as whole mount (FMNH 13774A, 1 slide).

g) One mature specimen (9mm X 5mm) as whole mount (FMNH 13774D, 1 slide).

h) One juvenile specimen (6mm X 4mm), as sagittal sections of the reproductive structures (FMNH 13774F, 6 slides).

i) an additional 16 specimens were examined

Etymology: named in honor of Dr. Robert Carney, Louisiana State University, chief scientist of the cruise on which the type specimens were collected.

Distribution and Habitat: To date, known from the type locality; on and in a wild wood fall.

Diagnosis: Prostatic vesicle interpolated and provided with two accessory prostatic vesicles, each bearing a stylet. The stylets merge to form a single copulatory organ. Large, glandular cells surround the male atrium. Female complex opens into the male atrium or very close to it. Cerebral eyes are in two rows. Tentacular eyes present.

Description

   External features: *Color--* in preserved specimens, the dorsal surface is whitish with brown to pink pigment, more concentrated in the region surrounding the pharynx. Numerous brown dots are disposed radially from the region of the pharynx toward the margin, corresponding to the ovaries and testes (Fig. 13A). Ventral surface is cream whitish, the uteri are visible as two dark lines located on either side of the pharynx (Fig. 13B). Probably the species is very pale pink when alive (Dr. J. Voight, pers. comm.).

*Form--*ovoid, somewhat tapered at the posterior end in some specimens. Length to width ratio of most specimens is from 1.4 to 2.2.

Tentacles- no evidence of tentacles.

Eyes-- two rounded clusters of tentacular eyes present on either side of the brain, they are separated by about 1 mm; each cluster contains 4 to 7 eyes. The cerebral eyes form two rows anterior to the brain, each containing 3 to 5 eyes (Fig. 14A). Marginal eyes are lacking.

Digestive system: a long ruffled pharynx, with about 7 pairs of folds, is positioned centrally (Fig. 13A) and occupies about 30 % of the total body length. The mouth is located at the posterior half of the pharynx. The main intestine branches toward the margin forming a highly anastomized digestive system. A median intestinal frontal branch is present extending dorsally over the bilobed brain.

Epidermis and body wall: a simple epithelium encloses the entire body (Fig. 15). Although cilia are present, they are not easily seen in histological sections. The thickness of the epidermis is 15 µm both on the ventral and the dorsal surfaces. Rhabdites are scarce and not very conspicuous either ventrally or dorsally. Commonly, polyclad rhabdites stain intensely pink, however, in this species they do not. Cyanophilous glands of a rounded shape are abundant throughout the entire epidermis. The dorsal epidermis carries only clusters of small rounded black pigment spots that are well-spaced and not very numerous. The basement membrane is difficult to distinguish from the base of the epidermis. The body wall is thicker ventrally (50 µm) with clear differentiation between circular, longitudinal and diagonal muscle layers (Fig. 15B). In contrast, the dorsal body wall is much thinner (20 µm) and the muscle layers are not well organized. Only a few weakly developed diagonal fibers and a layer of longitudinal muscles are evident. Beneath the epidermis and muscles of the dorsal surface,

concentrated brown pigment granules form a layer that runs parallel to the muscles and mix with some muscle fibers. No pigment was observed in the parenchyma.

Reproductive anatomy: *Gonopores--* are located at the distal posterior half of the body. The female gonopore is extremely close to the male gonopore (Fig. 14B) making it difficult to distinguish them in whole mount preparations. The uteri appear as two dark rows on each side of the pharynx (Fig. 13B).

Male reproductive system-- the male copulatory apparatus is characteristic for the genus. It is composed of two spermiducal bulbs connected ventro-anteriorly to the seminal vesicle. The seminal vesicle is a large oval organ (400 μm X 200 μm) positioned perpendicular to the body wall (Figs. 15A, 16). The interpolated prostatic vesicle is a complex organ lined by a smooth glandular epithelium. Two accessory prostatic vesicles connect to the prostatic vesicle proper at its distal end (Figs. 14B, 15A, 16). Each accessory prostatic vesicle bears a cuticular stylet (~175μm) which fuse to emerge as a single copulatory organ (Figs. 14B, 15A, 16). Surrounding the male atrium and the prostatic vesicle are several large glandular cells (Figs. 14B, 15B, 16). Each cell has a neck that penetrates the musculature of the prostatic vesicle wall to release its glandular content into the prostatic vesicle lumen or into the male atrium (Figs. 15B, 16). The deep and muscular atrium houses the copulatory organ (composed of fused cuticular stylets) and the distal portions of the prostatic organs (Figs. 15A, 16). Testes are located ventrally in the posterior part of the body.

Female reproductive system-- in most of the specimens, the female pore opens into or very close to the male atrium (Figs. 14B, 15A, 16). The simple female apparatus consists of a very short vagina that parallels the body wall (Figs. 15A, 16). The glandular cells of the cement glands surround the entire vagina (Fig. 15A). Lang's vesicle is absent. The

oviducts join the vagina dorsally, extend anteriorly, and connect to the uteri on each side of the pharynx (Fig. 16).
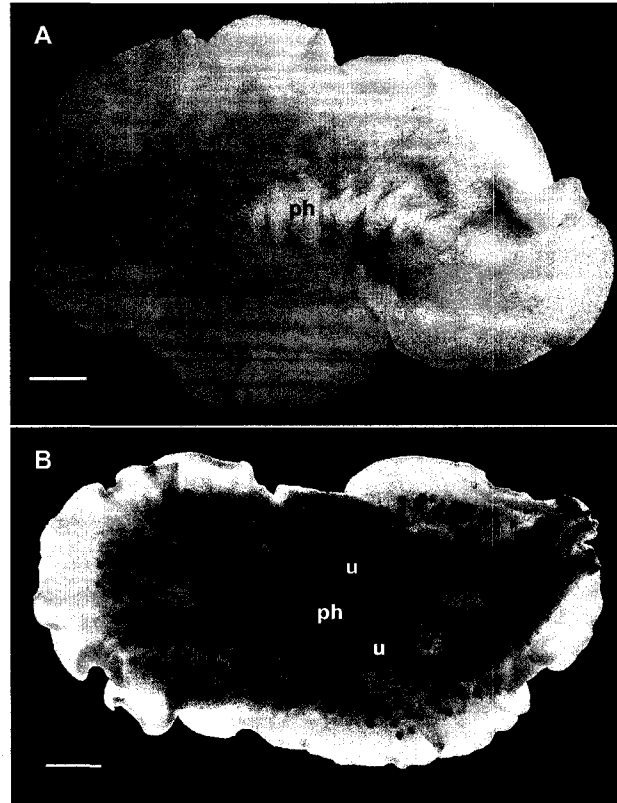
Figure 13. *Didangia carneyi*, sp. nov. A. Dorsal view of preserved specimen showing two clusters of tentacular eyes (arrow heads) and pharynx (ph). B. Ventral view of cleared whole mount, showing uteri (u), and pharynx (ph). Scale bars = 1 mm.
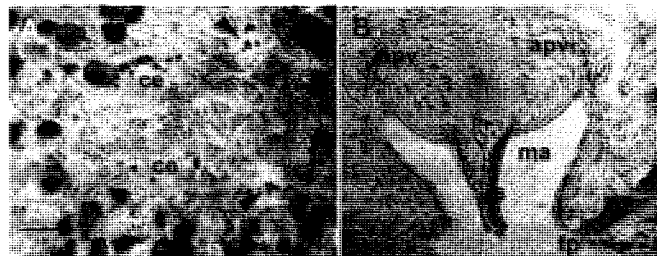
Figure 14. *Didangia carneyi*, sp. nov. A. Dorsal view of tentacular eye clusters (arrowheads) and two rows of cerebral eyes (ce) extending anterior. Scale bar = 250 μm. B. Male copulatory organ showing two accessory prostatic vesicles (apv) and stylet (s) entering the male atrium (ma); note female pore (fp) opening close to the male atrium. Scale bar = 50 μm.
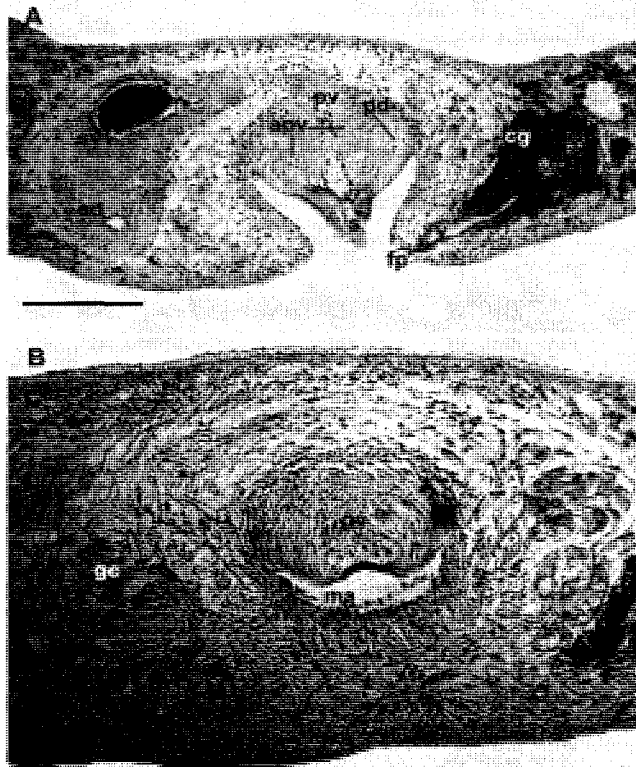
Figure 15. *Didangia carneyi,* sp. nov. A. Sagittal section through male and female reproductive systems, showing seminal vesicle (sv), ejaculatory duct (ed), prostatic vesicle (pv), accessory prostatic vesicles (apv), prostatic duct (pd), stylet (s), male atrium (ma), female pore (fp), vagina (v), and cement glands (cg). B. Sagittal section through prostatic vesicle, showing large glandular cells (gc), releasing secretions into prostatic vesicle (pv) or the male atrium (ma). Scale bars = 250 µm.
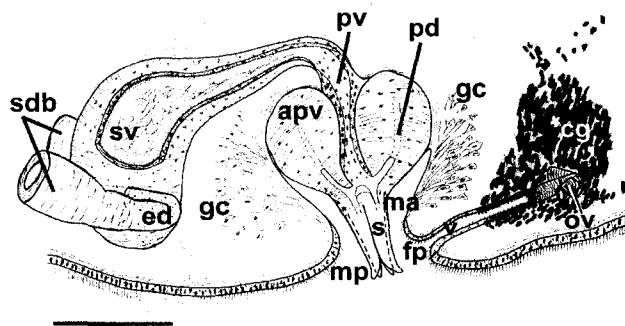
Figure 16. *Didangia carneyi,* sp. nov. Diagrammatic reconstruction of the male and female reproductive systems; accessory prostatic vesicle (apv); cg, cement glands; ejaculatory duct (ed); female pore (fp); gland cells (cg); male atrium (ma); male pore (mp); oviduct (ov); prostatic duct (pd); prostatic vesicle (pv); stylet (s); seminal vesicle (sv); vagina (v). Scale bar = 250 µm.

Taxonomic Remarks

A male copulatory apparatus located anterior to the male pore and directed posteriorly, a prostatic vesicle with a smooth glandular lining and no projection of the ejaculatory duct into its lumen, a ruffled pharynx, and two accessory prostatic vesicles all place the currently described species in the family Didangiidae (Faubel, 1983). Didangiidae contains only *Didangia mactanensis* (Faubel, 1983), and the family is defined based on characters of that species. A request to the Zoological Museum at the University of Hamburg, Germany for the original type material of *D. mactanensis* revealed that the type cannot be found (P. Stiewe, collections manager, pers. comm.).

The most conspicuous character of the family Didangiidae is the presence of a prostatic vesicle with smooth glandular lining and composed of two accessory prostatic vesicles. *Didangia carneyi* differs from the definition of the family, and from *D. mactanensis*, by the apparent presence of a common pore or gonopore, tentacular eyes and spermiducal bulbs. Yet, the most important character differentiating this new species is the orientation of the two accessory prostatic vesicles. In *D. mactanensis*, the accessory prostatic vesicles are situated laterally on both sides of the prostatic vesicle (perpendicular to the long axis of the body), whereas in *D. carneyi*, one accessory vesicle is located anterior to the prostatic vesicle and the other posterior to it (parallel to the long axis). The common gonopore, presence of tentacular eyes and spermiducal bulbs and the orientation of the accessory prostatic vesicles support the recognition of a new species but are not considered to be sufficient basis to erect a new family or genus. Among polyclads in general, only the cotylean family Prosthiostomidae and the acotylean Didangiidae have two accessory prostatic vesicles. In prosthiostomids though, multiple cuticular elements forming a copulatory organ are not found, and of course, by possessing a sucker, the family clearly belongs to the Cotylea.

Suborder: Cotyela Lang, 1884

Superfamily: Euryleptoidea Faubel, 1984

Family: Euryleptidae Lang, 1884

Genus: *Oligocladus* Lang, 1884

Species: *bathymodiensis* sp. nov. (Fig. 17-20)

Type Material and Locality:

a) Holotype: one mature specimen (8mm X 6mm) as sagittal sections of the reproductive structures (FMNH 13970, 7 slides) (USA: Lousiana, Gulf of Mexico, crewed submersible Johnson Sea-Link II from the Brine Pool NR-1 at Station VOIJ-JSLII-3521, coordinates: 27°00'28" N 91°16'45"W, water depth: 650 m). Collected on 14 August 2006.

b) Paratype: one mature specimen (8mm X 5mm) as whole mount (FMNH 13971, 1 slide); collected with the holotype.

Other Material:

a) one mature specimen (8.5mm X 5mm) as sagittal sections of the reproductive structures (FMNH 13776A, 6 slides); collected with the holotype.

b) one juvenile (3.5mm X 2mm) as whole mount (FMNH 13776D, 1 slide); collected with the holotype.

c) one mature specimen (8mm X 5mm) as whole mount (FMNH 13972, 1 slide); collected with the holotype of *Didangia carneyi*, (USA: Louisiana, Gulf of Mexico from a

wild wood fall at Station VOIJ-JSLII-3531, JSL Dive number 3531; coordinates: 27°44.09'N 91°14.49'W, water depth: 610 m). Collected on19 August 2006.

Etymology: Named for the deep-sea mussel *Bathymodiolus childressi*, Gustafson 1998 with which the holotype and many of the paratypes of this flatworm species was associated.

Distribution and Habitat: To date known from the type locality, a brine pool (NR-1) at 27°00'28" N 91°16'45"W and from a wild wood fall at 27°44'09"N 91°14'49"W, Louisiana, Gulf of Mexico, USA.

Diagnosis: Mouth anterior to the brain. A few cerebral eyes in two close clusters. Other minute eyes scattered over the pseudotentacles. About four pairs of main intestinal branches radiating from the main median intestinal branch. Ventral anal pore located posterior at the end of the main median branch of the intestine.

Description

External features: *Color--* in preserved animals, the ventral and the dorsal surfaces are whitish with sandy brown pigment distributed irregularly in the epidermis and concentrated at the tip of the tentacles. In some specimens, conspicuous white dots on both sides behind the pharynx are visible, corresponding to the uterine vesicles (Fig. 17A).

Form-- overall shape is oval in most specimens, somewhat tapered at the posterior end (Fig. 17A). Length to width ratios in all material examined were between 1.3 and 1.75. The dorsal surface is raised where the anterior tubular pharynx is located (Fig. 17A). The

pharynx occupies about 30% of the total body length. A prominent sucker is located in the center of the ventral surface posterior to the pharynx (Figs. 17B, 19B).

Tentacles-- A pair of conspicuous tentacles is present; widely separated (~1mm), long and blunt (Fig. 18) and formed by the elongation and folding of the anterior margin. Their shape is typical of that of the family Euryleptidae.

Eyes-- superficially this species appears to have only a pair of cerebral eyes (Fig. 18A). However, histological sections and cleared whole mounts revealed other small eyes immersed in the epidermis and the parenchyma and scattered in the cerebral and frontal regions, with a few scattered eyes on the tentacles as well (Fig. 18B).

Digestive system: the mouth is a small ventral opening located anterior to the brain (Fig. 19A). However, sometimes when the pharynx is extended, the mouth has a wide aperture. The cylindrical pharynx is typical for the family and is directed anteriorly. It connects to a wide median intestine that extends posteriorly, ending in a ventral aperture considered to be an anus (Fig. 19B). Four pairs of intestinal ramifications extend from the median intestinal branch and anastomize towards the margin (Fig. 17B).

Epidermis and body wall: a simple multi-ciliated epithelium surrounds the entire body. The cilia, readily visible in histological sections, are denser and longer on the dorsal surface. Although rhabdites are evident on both surfaces, they are arranged in dense packets in the dorsal epithelium and individually in the ventral epidermis. Also, the rhabdites are longer and more numerous dorsally than ventrally, making the dorsal epidermis taller (40 µm). Cyanophilous glands are present basally in the epidermis. There is not a significant difference in the thickness of the dorsal and ventral body wall

(15 µm). Muscle layers are not conspicuous and only a longitudinal layer is clearly visible.

Reproductive anatomy: *Gonopores--* are located in the anterior half of the body, ventral to the pharynx. The male gonopore is anterior to the female pore.

Male reproductive system-- small male system located posterior to the male pore and directed anteriorly. The elongated free prostatic vesicle (~500µm X 200µm) is positioned dorsally to the ejaculatory duct. The rounded seminal vesicle (450µm X 600µm) connects with the penis through a wide ejaculatory duct (Fig. 19C). The male atrium is deep in relation to the size of the whole male complex and houses a conical penis papilla that bears a cuticular stylet (Figs. 19C, 20). The testes are ventral.

Female reproductive system-- the female apparatus is very simple, with a pore leading into a short vagina (Figs. 19C, 20). No cement pouches are evident, but cement glands are present. The female gonopore is positioned about 1.4 mm from the male gonopore (Fig. 19C). About 9 pairs of uterine vesicles are present posterior to the pharynx (Fig. 17A).

Taxonomic Remarks

All species in the genus *Oligocladus* are characterized by the mouth positioned anterior to the brain (Hadenfeldt 1929). In some of the examined specimens of *O. bathymodiensis*, the mouth is positioned either just ventral or slightly posterior to the brain. This is due to a fixation artifact. Upon fixation, specimens will contract to some extent and in most euryleptids this contraction results in a curvature of the strongly

muscular pharynx and hence of the entire anterior end. This in turn, is responsible for

the artificial positioning of the mouth either slightly posterior or ventral to the brain.
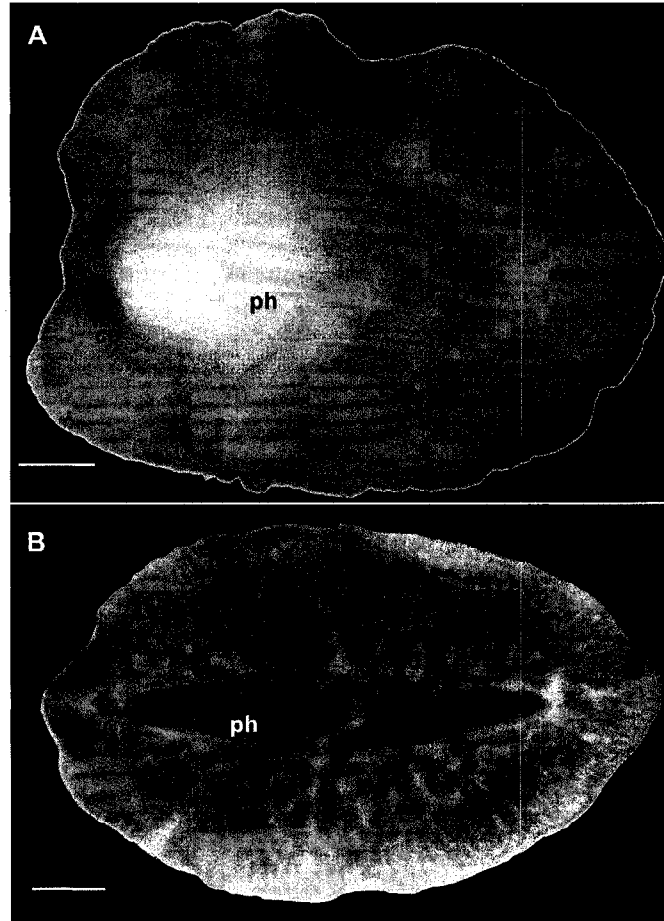
Figure 17. *Oligocladus bathymodiensis,* sp. nov. A. Dorsal view of preserved specimen, showing pharynx (ph), uterine vesicles (uv), and the position of cerbral eyes (ce). B. Ventral view of cleared whole mount, showing pharynx (ph), sucker (su), and uteri (u). Scale bars = 1 mm.

Figure 18. *Oligocladus bathymodiensis,* sp. nov. A. Anterior end of preserved specimen showing tentacles and cerebral eyes, scale bar = 100 μm. B. Sagittal section showing tentacular eyes (te), scale bar = 250 μm
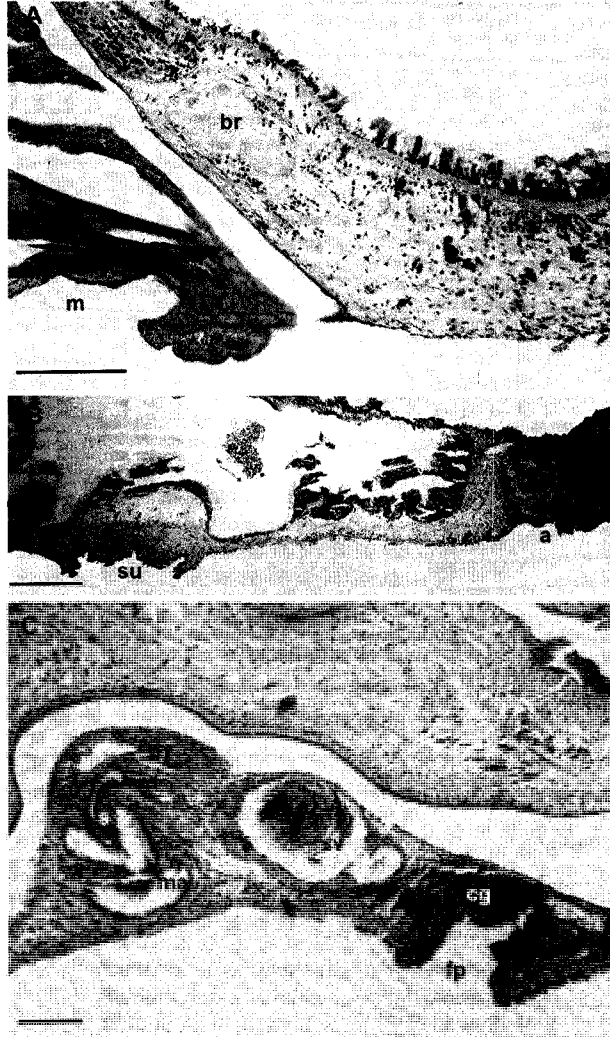
Figure 19. *Oligocladus bathymodiensis,* sp. nov. A. Sagittal section showing mouth opening (m) with protruded pharyngeal tissue (pt) and brain (br) located posterior to the mouth. B. Sagittal section with sucker (su) and ventrally opening anus (a). C. Sagittal section through the reproductive systems; cement glands (cg);ejaculatory duct (ed); female pore (fp); male atrium (ma); prostatic vesicle (pv); stylet (s); seminal vesicle (sv); vagina (v). Scale bars = 250 μm.
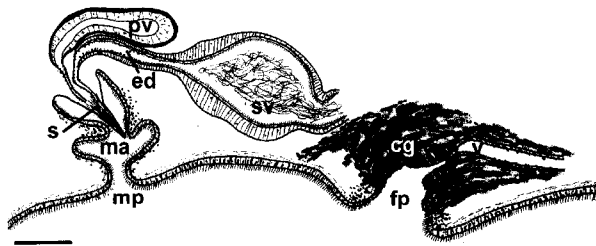
Figure 20. *Oligocladus bathymodiensis,* sp. nov. Diagrammatic reconstruction of the male and female reproductive systems; cement glands (cg), female pore (fp); male atrium (ma); male pore (mp); prostatic vesicle (pv); stylet (s); seminal vesicle (sv); vagina (v). Scale bar = 250 μm.

The main characteristics that distinguish *Oligocladus bathymodiensis* from its congeners, and especially from *O. voightae*, include the complete absence of an anterior median intestinal branch; this branch is present in *O. voightae* and is trifurcated in *O. sanguinolentus*. Secondly, a distinctive ventral position of the anus separates *O. bathymodiensis* from other *Oligocladus* species. An anus is a unique characteristic of the genus, however to date, in all known species it has been shown to open dorsally. A third unique characteristic is the presence of paired cerebral eyes, which actually consist of two distinct clusters of eyespots. *Oligocladus voightae* completely lacks cerebral eyes and *O. sanguinolentus* has two very well differentiated clusters of cerebral eyes. Finally, an auxiliary sperm storage vesicle connected to the seminal vesicle continues to be an exceptional character of *O. voightae* and is lacking in *O. bathymodiensis*.

Discussion

Members of the polyclad family Euryleptidae generally are quite common in shallow tropical waters, where they contribute to the colorful fauna of coral reefs (Newman and Cannon 1994a, 2000, 2002). However, *Oligocladus bathymodiensis* is now the third euryleptid species described from deeper waters, the other two being *O. voightae* Quiroga et al. 2006 and *Stygolepta hjalmari* Faubel, 1984. Two additional euryleptid specimens have been found in a trawl sample collected off the Pacific coast of Guatemala from about 110 m. Again, the worms were associated with wood-boring clams. Positive identification to species was not possible because of the conditions of the specimens. The two specimens are in the Benthic Invertebrate Collection at the Scripps Institution of Oceanography in La Jolla, California (Catalogue Number Pt 49). Hence, it appears that euryleptids may actually constitute a large component of the polyclad fauna of deeper waters.

A literature-based survey of the polyclad fauna of the Gulf of Mexico revealed 26 species, of which 4 species are endemic (Hooge and Newman, in press). The distribution of the other 22 species extends well into the Caribbean and in some cases even into the northwestern Atlantic. Sampling in these early studies however was biased towards the intertidal to shallow subtidal zones and regionally confined to Florida and Texas (Pearse 1938; Hyman 1940, 1954b, 1955a, b). This is the first report of two species of polyclads from depths greater than 600 m in the Gulf of Mexico.

Several species of acotylean polyclads are commonly referred to as "oyster wafers" or "oyster leeches," although they clearly are not annelids. This label is due to their voracious feeding on juvenile oyster spat and other commercially important bivalve species (Provenzano 1961; Webster and Medford 1961; Christensen 1973; Chintala and Kennedy 1993; Newman et al. 1993; Newell et al. 2000). Stylochids (*Stylochus frontalis*, *S. ellipticus*, *S. oculiferus*) are common in the Gulf of Mexico and either have been shown to prey directly on oysters or have been found in association with oyster shells and are thus suspected bivalve predators. It is interesting to note that at least one of the newly described species, *Oligocladus bathymodiensis* was found associated with beds of the mussel, *Bathymodiolus childressi*. Specimens of *D. carneyi* were collected from a wood-fall heavily bored by both teredinids and species of Xylophagainae. It is thought highly likely that the mussels and wood-boring bivalves represent a food source for the worms. A similar relationship has been described for two other deep-sea polyclads (Quiroga et al. 2006), which were only found on wood heavily colonized by wood-boring clams of *Xylophaga* (Voight 2007). Thus, the present study contributes not only to our knowledge of polyclad biodiversity but also increases our understanding of the ecology of deep-sea fauna.

CHAPTER V.

GROSS MORPHOLOGY OF THE CENTRAL NERVOUS SYSTEM OF
POLYCLAD FLATWORMS: A COMPARATIVE ANALYSIS[4]

Introduction

Typically, the central nervous system (CNS) of free-living flatworms consists of

an anterior brain from which several major nerve cords extend. The brain may range

from a nervous layer over the statocyst to a sizable, well-defined ganglion that may be

enclosed by a thin, distinct capsule. In most turbellarians, the main nervous system is

located immediately below the subepidermal musculature forming a submuscular

nervous plexus (Hyman 1951). Within this plexus, a varying number of major longitudinal

cords can be discerned. The number, position, and arrangement of these major nerve

cords vary dramatically from one taxonomic group to the next, although commonly the

ventral cords are more strongly developed than other cords.

Reisinger (1925) defined a standard orthogonal pattern for many turbellarians.

This pattern consists of longitudinal nerve cords that are connected at right angles along

their lengths by transverse commissures, forming a ladder-like arrangement. Hence, the

traditional view of the turbellarian CNS includes the orthogon plus the brain or cerebral

ganglion. Although the orthogon is considered homologous among all flatworms, many

different types (i. e., position and number of cords, different contact points with brain,

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[4] Submitted to Zoologischer Anzeiger. June 2008.

thickness of cords) exist. Reuter et al. (1998) have developed terminology to distinguish main cords from minor ones. According to these authors, main cords have strong roots in the brain, consist of wide fiber bundles, and have numerous neurons that show immunoreactivity of serotonin and catecholamines. In the past, much confusion had been generated by describing main cords in lateral, ventral, dorsal, ventro-lateral, dorso-lateral, or marginal positions. In contrast to earlier views (Reisinger 1925) which include the brain plus the orthogon in the definition of the turbellarian CNS, Reuter et al. (1998) only include the brain plus one pair of main nerve cords in their definition. Using the above criteria, the authors propose homology of main nerve cords in all flatworms (regardless of the actual position of the cords) thus, providing a testable phylogenetic framework. To date, they have shown homology of main cords for several turbellarian orders; however, no such studies have been performed in polyclads.

Among flatworms, polyclads have complex brains, showing histological differentiation similar to that found in annelids, mollusks and some arthropods (Bullock and Horridge 1965). The CNS of *Notoplana acticola* (Boone 1929) consists of an anterior, bilobed ganglion that integrates all activities of the worm (Bernardo et al. 1977; Koopowitz 1973). This brain is enclosed by a capsule and is composed of an outer rind of cells and an inner core of fibers, formed by a variety of neuron types with different properties (e.g., bipolar, multipolar, decussation, coupling) (Koopowitz 1986). At the front of each brain lobe and located outside the capsule, are two groups of sensory ganglion cells (globuli cell masses, Körnerhaufen) that are of granular appearance.

Studies on *Stylochoplana maculata* (Quatrefages 1845), *Notoplana atomata* (Hadenfeldt 1929), *Planocera* (Lang 1879), and *Gnesioceros* (Hyman 1951) all show that a number of paired nerve cords radiate from the brain. The first two pairs are directed forward (anterior and anterior dorsal nerves), the third and fourth pairs (lateral

and lateral dorsal nerves) are directed laterally from the brain, and the last two pairs (posterior and posterior dorsal nerves) are directed posteriorly. These nerves soon branch repeatedly towards the margin, becoming more delicate and forming anastomosing plexi containing multipolar and bipolar neurons (Koopowitz 1974, 1986).

Hence, it appears that the polyclad nervous system is very different from the orthogon typical for many flatworms. In fact, these differences led Minichev and Pugovkin (1979) to conclude that the polyclad nervous system is not homologous to that of other flatworms. As of yet, no comparative studies of the polyclad CNS have been performed. In this study, I describe the gross morphology of the CNS for 12 different polyclad species representing 11 families. I apply the morphological criteria of Reuter et al. (1998) to distinguish main nerve cords from secondary nerve cords. Based on my results, I establish three different categories of polyclad CNS and show their potential phylogenetic value. Although ventral nerve cords other than the main cords also are described, they are included for descriptive purposes only.

Materials and Methods

Specimens representing 11 species of polyclads were collected from different sites in the Caribbean. In addition, a deep-sea specimen of *Anocellidus profundus*, from the North Pacific was also included in my analysis (specimen courtesy of Dr. Janet Voight, Field Museum of Natural History, Chicago, Illinois, USA). The Caribbean specimens were hand collected in the littoral zone and by SCUBA from the sublittoral zone from under rocks and coral rubble. Live animals were measured (measurements given as length mm x width mm) and photographed in vivo in the lab, fixed on frozen

10% buffered formalin (method modified after Newman and Cannon 1995) and preserved in 70 % ethanol.

For species identifications, a segment containing the reproductive structures was dissected from one specimen of each species. This segment was embedded in paraffin, sagittally sectioned at 5-7 μm with an AO 820 Spencer microtome, and stained with hematoxylin and eosin. Sections were mounted in Permount on positively-charged glass slides.

For the examination of the nervous system, a second specimen of each species was embedded in paraffin in its entirety, longitudinally sectioned at 7-10 μm, and stained. To determine the optimal staining method for nervous structures, specimens of *Melloplana ferruginea* was stained with hematoxylin and eosin, and with Milligan Trichrome Staining (Presnell and Schreibman 1997). No significant differences were found between the two methods, and either method allowed for the description of the gross anatomy of the CNS. Thus, the remaining specimens were stained using Milligan Trichrome Staining (see appendix A).

Milligan Trichrome Staining involves the deparaffinization of sections and their transfer to 95% alcohol followed by treatment with Mordant in 3% potassium dichromate-hydrochloric acid solution for 5 minutes. Following a distilled water rinse, sections were stained in acid fuchsin for 8 minutes. After another distilled water rinse, they were placed into 1% phosphomolybdic acid for 2 minutes and then into Orange G for 5 more minutes. After a final rinse with distilled water, the sections were treated with 1% aqueous chloridric acid for 2 minutes, stained in Fast Green for 5 minutes, treated with 1% acetic acid for 3 minutes and then rinsed in 95% alcohol and dehydrated. Finally, the sections were cleared with Histoclear and mounted in Permount.

When required for interpretation, cross and sagittal sections were obtained and stained as well. Furthermore, whole mounts of each species were prepared, by dehydrating the specimens in a graded alcohol series, cleared with Histoclear and mounted in Permount.

Species identifications and taxonomic assignments follow Faubel (1983, 1984a), a classification system that is based on characters of the reproductive system. A second classification system proposed by Prudhoe (1985) is based on plastic characters of eye arrangement. Although some taxonomic entities generated by Faubel (1983, 1984a) are based on highly subjective characters (e. g., thickness of the lining of the prostatic vesicle), the majority of characters associated with the reproductive system are stable.

Results

Overall Gross Morphology (Fig. 21)

In all examined species, the overall arrangement of the CNS consisted of an anteriorly located brain from which several branches of nerve cords extended in different directions as described above. The encapsulated brain was clearly bilobed but depending on the species, to differing degrees. At least four cell types could be discerned (Fig. 21). Type I cells were large ganglion cells with big nuclei. These cells were located mostly in the posterior part of the brain. Type II cells were globuli cells which are characterized by a highly reduced cytoplasmic content and big nuclei rich in chromatin. They occupied mostly the anterior rind of the brain and formed the external globuli cell masses. Type III cells were of medium size, located mainly in the lateral parts along the periphery of the brain. Type IV cells were small of elongated shape, and

located sporadically along the nerve tracts, especially at bifurcations; a few were found in the posterior rind of the brain (Fig. 21B).

Two main ventral thick nerve cords were consistently present. These always extended posteriorly, whereas most other nerve cords were radially distributed in the anterior part of the animal. Several thinner commissures of different arrangements connected the major nerve cord branches. A dorsal diffuse nerve net was found in most species examined.

Here I propose three categories based on the position of the brain, the thickness of the main nerve cords, the shape of the cords in cross sections, the number and position of the nerve cords in the body, and the tissue surrounding the nerve cords. I chose a representative model for each category and assigned the 12 examined species to these categories. Category I is described extensively, whereas for the remaining categories, I emphasize main differences only.
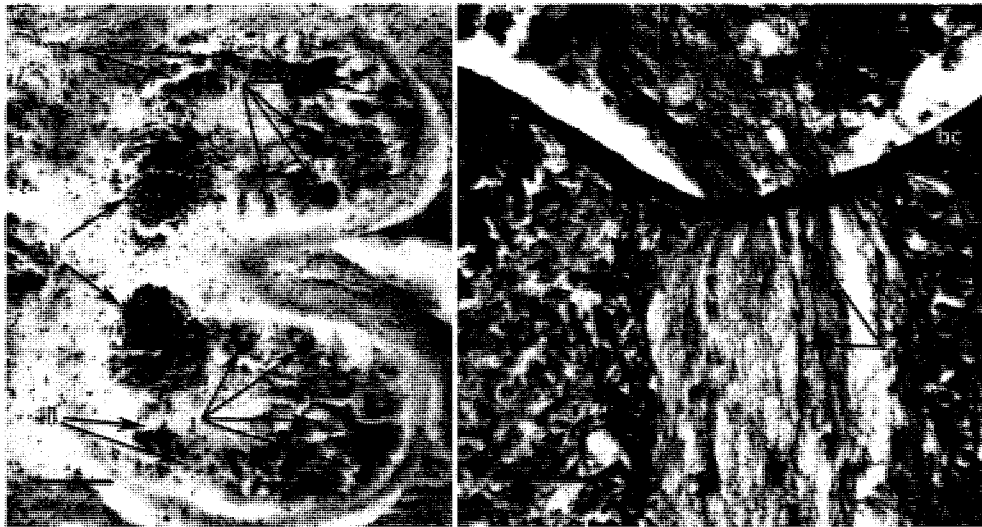
Figure 21. Longitudinal sections of the brain and nervous branches showing different types of cells distinguishable at the light microscopic level in *Styloplanocera fasciata*. A. Type I large ganglion cells with big nuclei, type II globuli cells, type III cells of medium size. Scale bar = 75 μm. B. Type IV small cells with elongated shape located along the nerve tracts. Scale bar = 25 μm. Brain (b); brain capsule (bc).

<u>**Category I CNS**</u> (Figs. 22 - 24)

Definition: species in this category had large sized encapsulated brains and

thick main nerve cords that were dorsoventrally flattened and completely submersed into

the parenchyma. Cerebral eyes were located in the vicinities of the brain but never

dorsal to the brain; tentacular eyes were found on each side of the brain. The globuli cell

masses were well defined (Fig. 22).
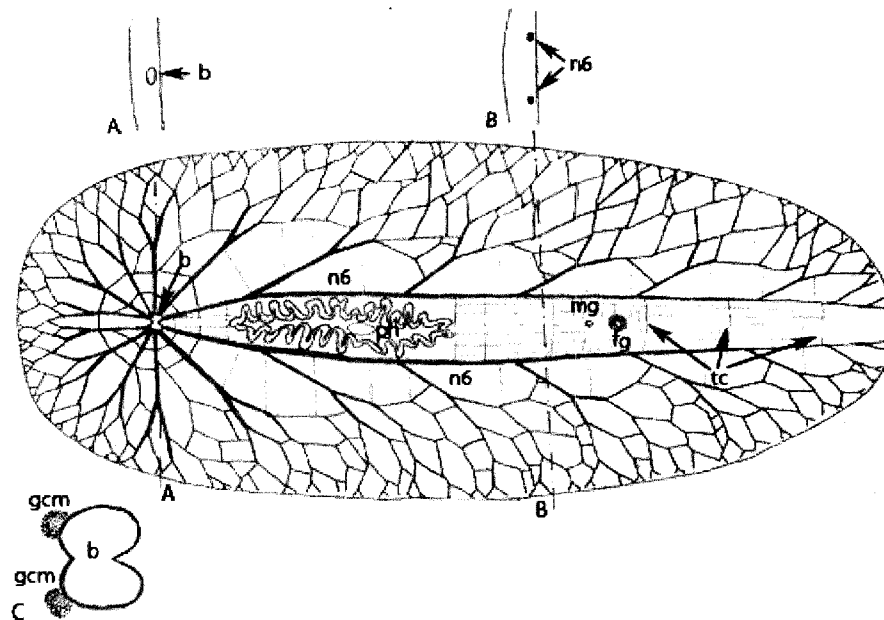
Figure 22. Schematic representation of the CNS for Category I. A. Cross section at the level of the brain. B. Cross section of the n6 nerve pair. C. Detail of the brain. Brain (b); female gonopore (fg); globuli cell masses (gcm); male gonopore (mg); major nerve cords (n6); pharynx (ph); transverse commissures (tc).

With one exception, the species in this category are all acotyleans (i. e.,

polyclads lacking a ventral adhesive disk, Lang 1884): *Styloplanocera fasciata*

(Gnesioceridae), *Melloplana ferruginea* (Pleioplanidae), *Armatoplana lactoalba*

(Stylochoplanidae), and *Phaenocelis medvedica* (Cryptocelidae), *Anocellidus profundus*

(Anocellidae), *Idioplana atlantica* (Pseudostylochidae), and *Pericelis cata* (Cotylea: Pericelidae).

Category I Model Species: *Styloplanocera fasciata*

The CNS of *Styloplanocera fasciata* was completely submersed in the parenchyma. The cerebral ganglion was enclosed by a capsule, and was located between the tentacles at about 1/5 to 1/6 of the total body length from the anterior margin. Cerebral eyes were scattered, close to the brain with a few just anterior to it, forming two distinct elongated clusters; additionally a few were located just posterior to the brain (Fig. 23A). No cerebral eyes were located directly above the brain capsule. The tentacles were covered with eyes along their entire lengths (Fig. 23A).

A dorsal view of whole mounts and longitudinal sections revealed very conspicuous depressions, which divide the brain into two lobes. The posterior depression was deeper than the anterior one. The brain was dorsoventrally flattened and in mature (~24 mm long) preserve specimens, it was about 525 µm wide, 475 µm long and 275 µm tall (Fig. 23A). In sagittal sections, it was possible to recognize the two lobes of the brain in the dorsal portion. A median intestinal branch extending anteriorly over the middle portion of the dorsal side of the brain, may give the appearance that the brain consists of two individual capsules (Fig. 23B). However, the lobes were very well connected ventrally (Fig. 23C).

From the brain capsule, six pairs of ventral nerve cords branched out (Fig. 23D). The brain capsule did not extend along the branches; rather it appeared that the nerve cords perforated the capsule. However, the nerve cords were covered by a sheath. The first four pairs innervated the region of the animal anterior to the brain in a well-defined

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radial pattern. The first pair (n1) radiated anteriorly (0°) and the remaining three pairs at angles of 30° (n2), 60° (n3) and 90° (n4) with respect to the longitudinal axis of the worm. The other two pairs (n5, n6) innervated the posterior region of the worm in angles of approximately 135° and 180°, respectively (Fig. 23D).

Some nerve cords shared roots as follows: n1/n2 and n5/n6; n3 and n4 did not share the same root but very close to the brain, n4 was connected with the root of n5 by a commissure (Fig. 23D). More distant from the brain, the nerves of the first four pairs subdivided into dichotomous branches toward the margin, where they became thinner and connected with other nerve fibers originating from the proximal nerve cords. Thus, these nerve fibers formed polygons (Fig. 22). Thereafter, they divided into a complex marginal net. Transverse commissures formed connections between the first four nerve cords. Pairs n5 and n6 innervated the region of the worm posterior to the brain. Pair n5 divided into dichotomous branches innervating the region from the level of the brain to the middle of the pharynx, and pair n6 started dividing where the pharynx began, innervating the remainder of the animal. All nerve cords were dorsoventrally flattened and in transverse sections, appeared oval (Fig. 22). The nerve cords of the n6 pair were the thickest ones with a diameter of about 250 µm, identifying them as the main nerve cords for species in this category. The diameters of the remaining nerve cords varied from 125 µm to 225 µm. Pair n6 extended the entire length of the worm, lying parallel on either side of the pharynx and becoming thinner posteriorly. Along their lengths, the two nerve cords were connected by transverse commissures and eventually, they divided into very thin branches, forming different plexi, such as the pharyngeal and reproductive plexi (Fig. 22).

Two groups of sensory ganglion cells (globuli cell masses, Körnerhaufen) of granular appearance were located external to the brain capsule between the roots of

nerve cord pairs n2 and n3 (Figs. 22C, 23E). Two additional sensory groups were present in the interior of the brain, located more dorsal than the exterior ones (Fig. 23F). This characteristic feature was common to all species in the category, except for *Pericelis cata*, in which four well-developed globuli cells masses were located above the roots of n2, n3, and n4 (Fig. 23G).

The interior of the brain was composed mainly by neurites and some perikarya. Within the brain were fibers forming different types of nerve tracts, which gave rise to the nerve cords or to the connections to the globuli cell masses (Fig. 24A- C). The neuropile was formed by a very dense mass of neurites and fibers but no cell bodies.

A few differences were observed among the species of this category. In most species, the brain was bilobed, but in those species where a median intestinal branch is absent or located distant from the brain, the brain appeared completely oval (e.g., *Idioplana atlantica*) or only slightly bilobed (Fig. 24D). The external globuli cell masses were present in all species examined in this category (Fig. 24D-G), however internal ones were evident only in *S. fasciata* that had been stained with hematoxylin and eosin. (Fig. 23F).

The number of main branches leaving the brain was constant in all the species of this category, but the degree of branching was variable. In species of elongate body form, (e. g., M. *ferruginea*, S. *fasciata*, A. *lactoalba* and P. *medvedica*), the pharynx is elongated and narrow, in which case the nerves of the n6 pair ran parallel to along each side of the pharynx (Fig. 24I). On the other hand, in species of oval body forms (e. g., *I. atlantica* and A. *profundus*) the pharynx was thrown into folds and hence, the nerves from the n6 pair ran ventral to the pharynx weaving among the pharyngeal folds (Fig. 24H).
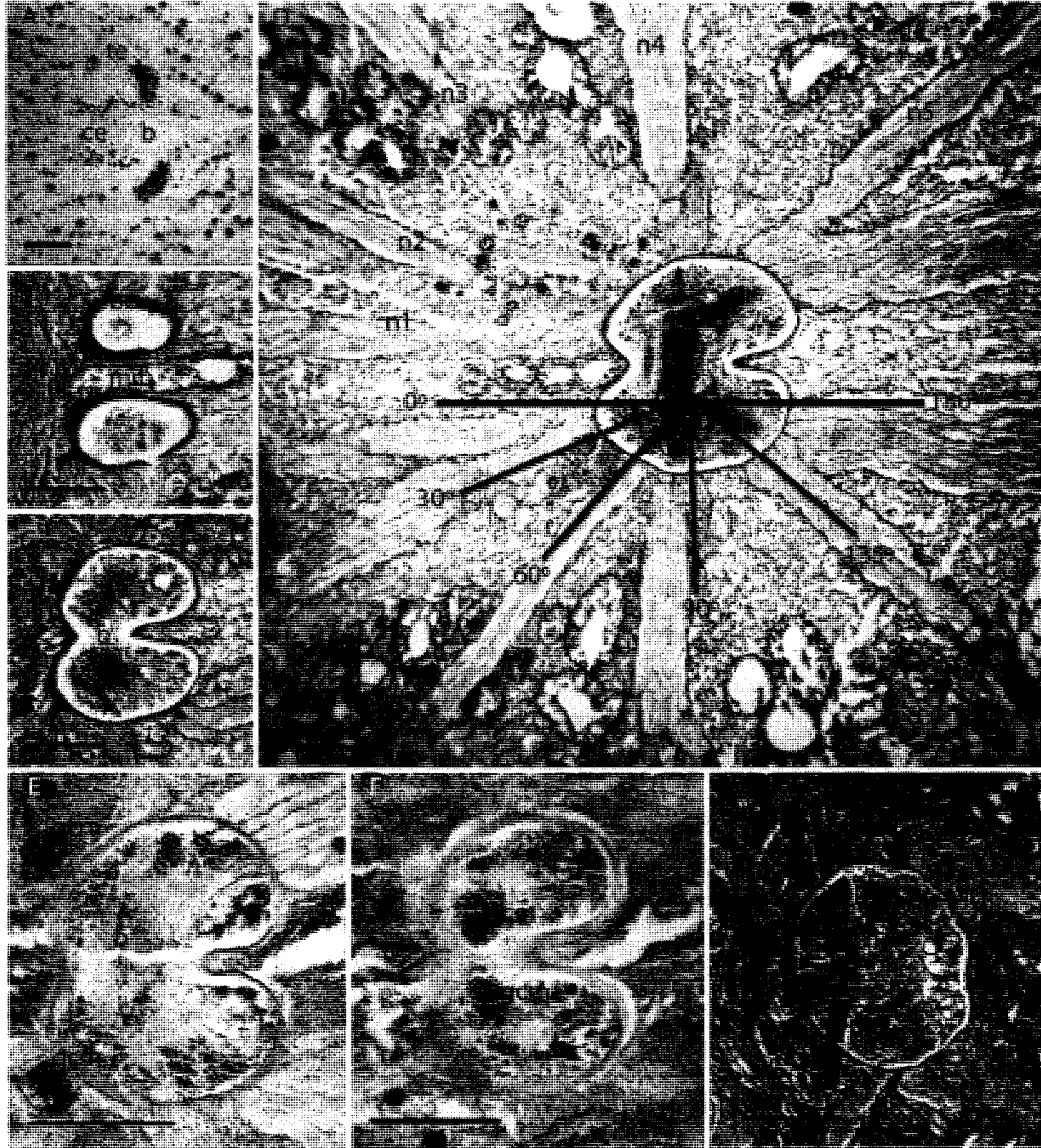
Figure 23. Category I species. A. Whole mount of *Styloplanocera fasciata*, showing the brain and tentacles. Scale bar = 500 µm. B. Section of the brain of *S. fasciata* trough its dorsal region. Scale bar = 250 µm. C. Section of the brain of *S. fasciata* trough its ventral region. Scale bar = 250 µm. D. General section showing the distribution of the ventral nerve cords of *S. fasciata*. Scale bar = 250µm. E. Section though the ventral region of the brain of *S. fasciata*, showing the external globuli cell masses. Scale bar = 250 µm. F. Section through the dorsal region of the brain of *S. fasciata*, showing the internal globuli cell masses. Scale bar = 250 µm. G. Section though the ventral region of the brain of *Pericelis cata*, showing its four external globuli cell masses. Scale bar = 250 µm. Brain (b); brain capsule (bc); cerebral eyes (ce); globuli cell masses (gcm); nerve cords (n); major nerve cords (n6); median intestinal branch (mib); tentacular eyes (te).

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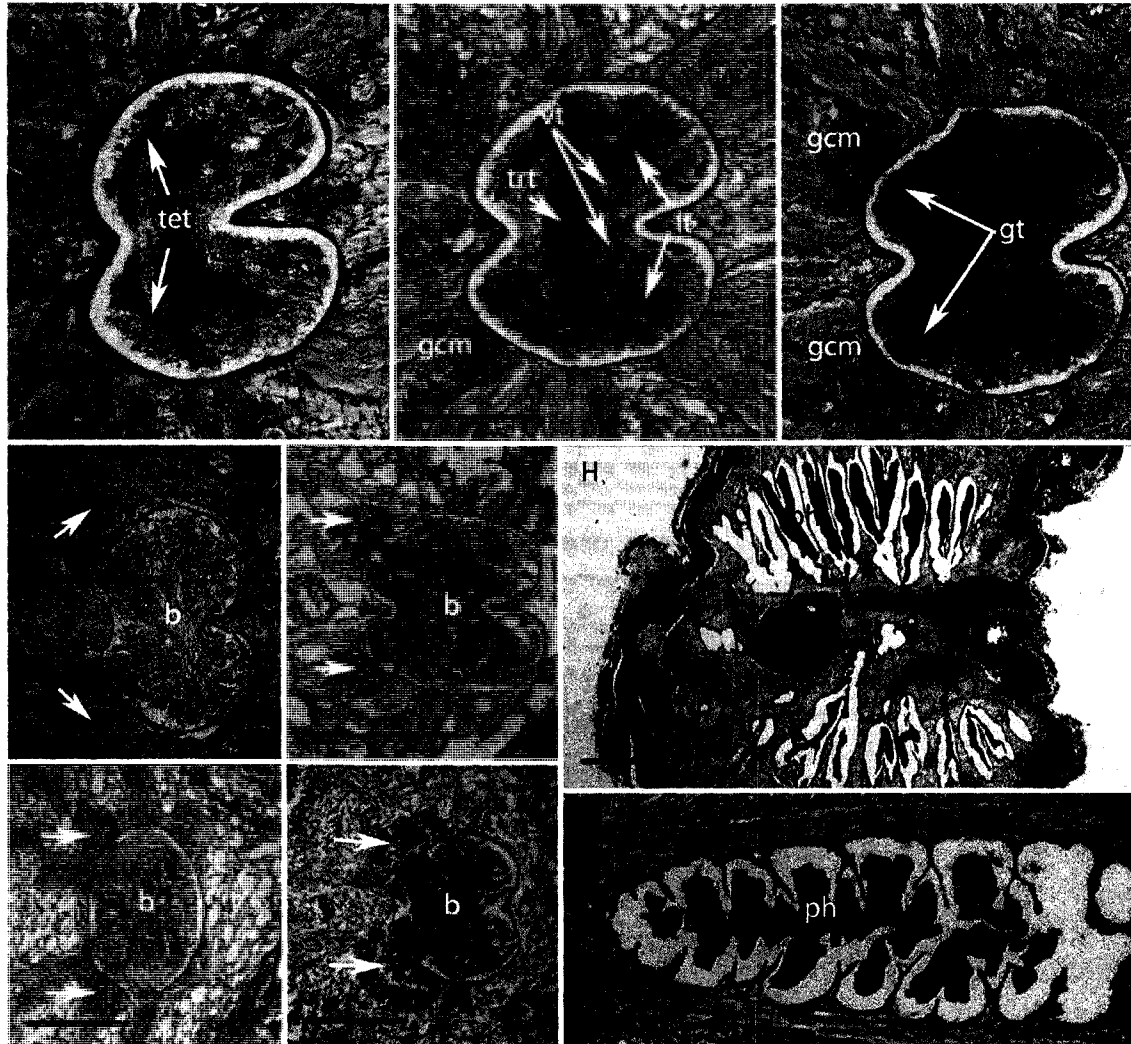
Figure 24. Category I species. A-C. Sections through the brain of *Styloplanocera fasciata* showing perikarya and different tracts. Scale bar = 250 μm. D-G. Sections of the brains from species showing different degrees of bifurcation. Arrows indicate external globuli cell masses. Scale bar 250 = μm; D. *Anocellidus profundus,* E. *Armatoplana lactoalba,* F. *Idioplana atlantica,* G. *Phaenocelis medvedica.* H- I. Differences in the distribution of the n6 nerve pair (indicated by arrows) in elongated and oval shapes polyclads. Scale bar = 500 μm; H. Oval shape *I. atlantica,* I. Elongated shape *Melloplana ferruginea.* Globuli cell masses (gcm); globuli cells masses tracts (gt); longitudinal tracts (lt); pharynx (ph); tentacular tracts (tet); transverse tracts (trt); vertical tracts (vt).

Category II CNS (Figs. 25 and 26)

Definition: Species in this category had a small brain, which was only slightly or

not at all bilobed. The main nerve cords were thin, dorsoventrally flattened, and

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completely submersed in the parenchyma. The cerebral eyes were located in the vicinity

of and/or above the brain; tentacular eyes were located on pseudotentacles, if present.

The globuli cell masses were poorly defined (Fig. 25).
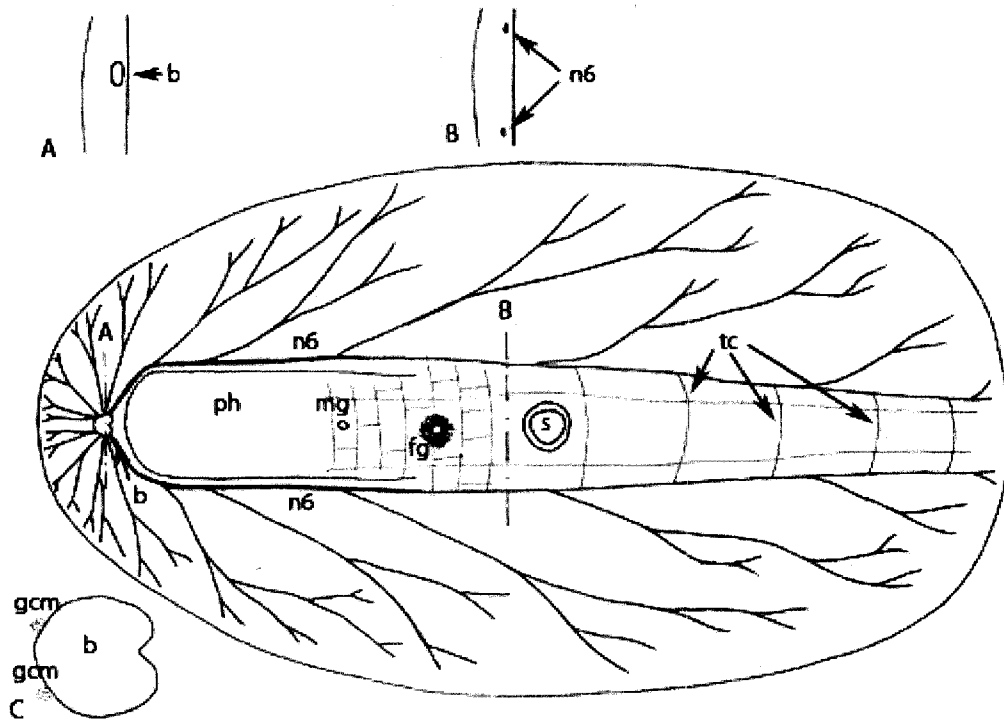
Figure 25. Schematic representation of the CNS for species of Category II. A. Cross section at the level of the brain. B. Cross section of the n6 nerve pair. C. Detail of the brain. Brain (b); female gonopore (fg); globuli cell masses (gcm); male gonopore (mg); major nerve cords (n6); pharynx (ph); sucker (s); transversal commissures (tc).

The species in this category are Cotylea (i. e., polyclads with a ventral sucker,

Lang 1884): *Enchiridium periommatum* (Prosthiostomidae), *Pseudoceros bolool, P.*

bicolor (Pseudocerotidae), and *Maritigrella crozieri* (Euryleptidae).

Category II Model Species: *Enchiridium periommatum*

In general terms, the CNS of *Enchiridium periommatum* was very similar to the

one described in Category I. The cerebral ganglion consisted of a complex of neurites

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and neuronal bodies enclosed by a thin capsule. It was located anteriorly, at about 1/8 of the total length of the body. In this particular species, the cerebral eyes were grouped into two clusters mostly in front of the brain but in the other species of this group the cerebral eyes formed a single cluster directly above the brain (Fig. 26A-B).

The brain was only slightly bilobed, having a shallow depression in its posterior part. However, in some species such as *Pseudoceros bicolor* and *P. bolool* it was not bilobed at all (Fig. 26D- F). As in *Melloplana ferruginea* (a species of category I), the brain capsule appeared to be of the same composition as the basal membrane. In a mature, preserved specimen of *E. periommatum* (19mm x 9mm) the brain was about 550µm wide, about 525µm long and about 300µm tall. All species in this category were characterized by having a small brain in relation to their body size as compared to species of Category I.

The number of main nervous branches exiting the brain was the same as for species in Category I. Six pairs radiated out but at slightly different angles (Fig. 26C). The main nerve cords of *E. periommatum* seemed not to be covered by a sheath and in other species of this group, if such a sheath existed, it was not very well defined. As in *Styloplanocera fasciata* n1, n2 and n5, n6 shared the same roots, but a commissure close to the brain connecting n4 and n5 was not evident. The arrangement and dichotomous branching of the main nerve cords was basically the same as that observed for species in Category I. However the ramifications had fewer transverse commissures connecting them (Fig. 25). The two nerve cords of the n6 pair ran parallel to the pharynx and were connected by thin transverse commissures along the entire length of the body; this was very conspicuous in *Maritigrella crozieri* (Fig. 26G). Because nerve pair n6 always was the thickest with a diameter of about 90µm, it was identified as the main nerve cords. The

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diameters of the remaining nerves cords varied from 35 μm to 70 μm.
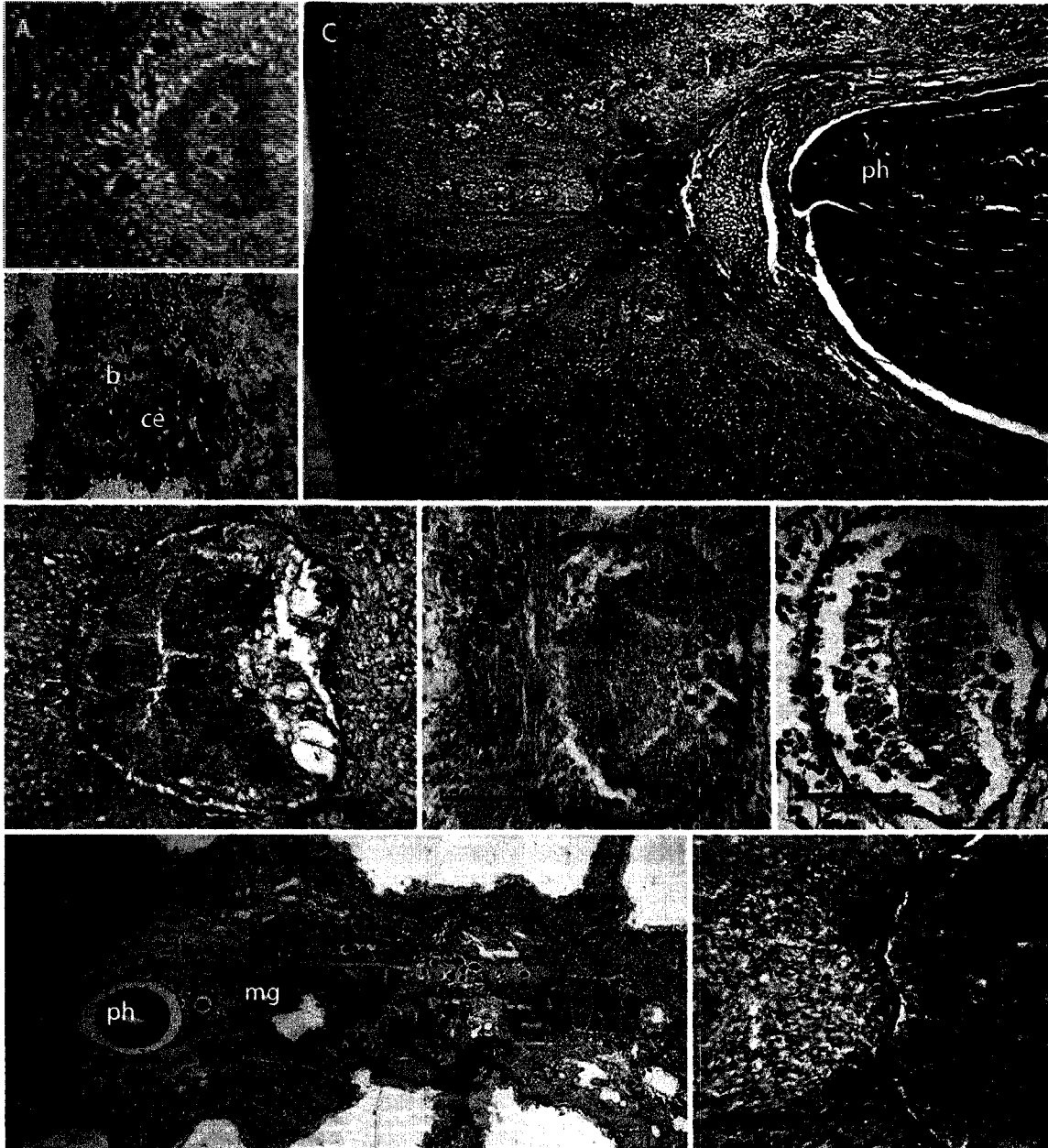
Figure 26. Category II species. A. Arrangement of the cerebral eyes in *Enchirium periommatum*. Scale bar = 100 μm. B. Arrangement of the cerebral eyes in *Pseudoceros bicolor*. Scale bar = 100 μm. C. General section showing the distribution of the main ventral nerve cords in *E. periommatum*. Scale bar = 250μm. D-F. Brain shapes. Scale bar 100 = μm. D. *E. periommatum*. E. *Pseudoceros bolool*. F. *P.bicolor*. G. Ventral nerve cords connected by transverse commissures in *Maritigrella crozieri*. Scale = bar 1mm. H. Anterior portion of the brain in *E. periommatum,* showing the poor development of the globuli cell masses. Scale bar = 100 μm. Brain (b); cerebral eyes (ce); female gonopore (fg); globuli cell masses (gcm); mouth (m); male gonopore (mg); nerve cords (n); pharynx (ph); transversal commissures (tc); ventral nerve cords (vnc).

The groups of globuli cell masses were not very well defined. In most species,

they consisted of few and scattered cells above the roots of n1 and n2 (Fig. 26H). No

interior globuli cells masses were observed in any of the specimens examined,

otherwise the interior histology of the brain was very similar to the one described for

Category I.

Category III CNS (Fig. 27 and 28)

Definition: The category includes species with a brain that is located very

anteriorly in the body (Fig. 27A). The brain and main nerve cords are submersed among

the longitudinal body wall muscles. The main nerve cords are thick and not flattened in

cross section (Fig. 27B). Cerebral eyes are scattered above and in the vicinity of the

brain. Tentacular eyes are located on true marginal tentacles. Globuli cell masses are

poorly defined (Fig. 27C).

Figure 27. Schematic representation of the CNS for Category III species. A. Cross section at the level of the brain. B. Cross section of the ventral nerve cords. C. Detail of the brain. Brain (b); female gonopore (fm); globuli cell masses (gmc); longitudinal muscles (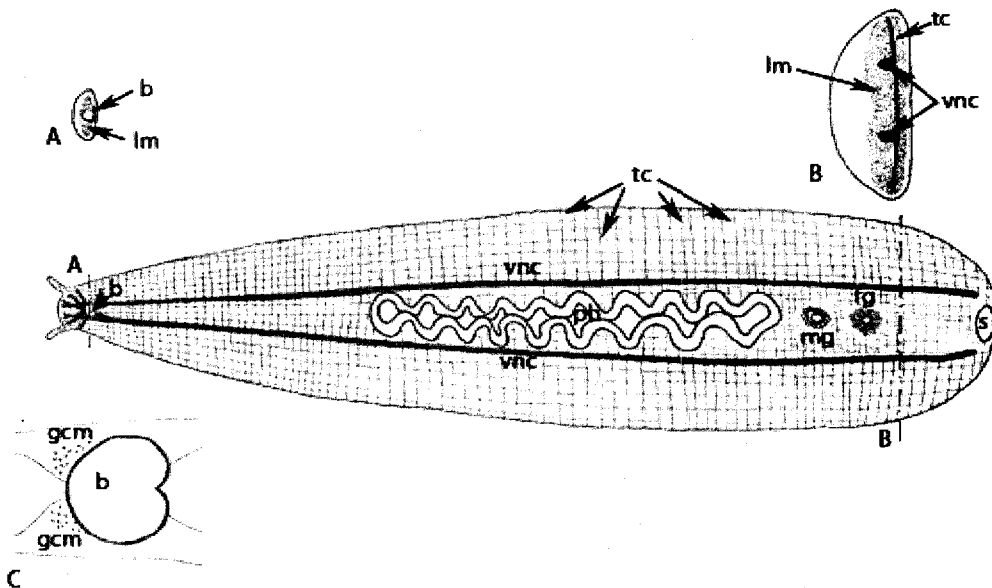lm); male gonopore (mg); major nerve cords (n6); pharynx (ph); s sucker; transversal commissures (tc); ventral nerve cords (vnc).

The sole species examined in this category belongs to the Boniniidae, a family currently classified in the Cotylea: *Boninia divae*.

Model Species for Category III: *Boninia divae*

The longitudinal musculature of *Boninia divae* was greatly developed, and the brain and main nerve cords were completely surrounded by longitudinal muscle fibers (Fig. 28A, B). This is strikingly different from the arrangement found in other polyclad species. Furthermore, neither the brain nor the main nerve branches were flattened. The brain was about 175µm in diameter and was located very anteriorly (about 1/10 in relation to the total body length). The main nerve branches were rounded in cross section, and in a mature specimen (18 mm x 4 mm), measured about 100 µm in diameter. The cerebral eyes were scattered above and in the vicinity of the brain. Tentacular eyes were located along the base of the true marginal tentacles (Fig. 28C).

The brain was not bilobed but it had a slight depression on its posterior part (Fig. 28D, E). The brain capsule was very well defined and enclosed a core of nervous fibers and a few peripheral cell bodies (Fig. 28D). At the light microscopic level, it was difficult to distinguish differences in cell types. However, numerous cells similar to type I cells were congregated in the posterior and ventral parts of the brain. A large number of cells similar to type II were concentrated anteriorly and dorsally (Fig. 28E). Type II cells were also found exterior to the brain, some of them scattered anterior to the brain, forming very rudimentary globuli cell masses (Fig. 28E).

A pair of ventral nerve cords (corresponding to n6 pair of other species) ran along the entire length of the body, parallel to each side of the pharynx (Fig. 27). At the anterior margin, this pair of nerve cords appeared to be fused ventrally with the brain.

There were no clear perforations of the brain capsule by these nerves cords. At the level of the brain and anterior to it, numerous nerve branches extended from the main ventral nerve cords, some of them fused towards the margins forming a radial pattern (Figs. 27 and 28G). In addition, about 7 to 10 thinner branches ran parallel to the main ventral nerve cords, and all of them including the main ventral nerve cords, were connected by transverse commissures that crossed from one side of the body to the other (Fig. 28F, H). This entire ventral plexus formed a very consistent orthogonal pattern from which some other plexi, such as the reproductive plexus originated (Fig. 28F, I).

At least three other pairs of nerve cords left the brain dorsally (Fig. 28J). These fibers subdivided into dichotomous branches, innervating the portion of the animal anterior to the brain, including the marginal tentacles. It is worthwhile to mention that a very diffuse, submuscular dorsal plexus was connected with the ventral plexus (Fig. 28K). All nerve cords were surrounded by a thin sheath (Fig. 28L).

Discussion

The CNS of all polyclad species examined in this study display the same overall configuration described in other studies (Hadenfeldt 1929; Hyman 1951; Bullock and Horridge 1965; Minichev and Pugovkin 1979). An anterior encapsulated brain, six pairs of ventral nervous branches, and the typical radial pattern forming a network seem to be constant features.

Minichev and Pugovkin (1979) contend that the nervous system in polyclads is not homologus with that of the other flatworms. Contrary to their assertion, I identified a pair of distinct main nerve cords (n6) that originated from an anteriorly located brain in all

species examined. The position, arrangement, and composition of the brain and main nerve cords allow for homologizing the components of the polyclad CNS with the CNS of other flatworms. Hence, the polyclad CNS corresponds to the orthogonal pattern common in other flatworms, in which two major longitudinal nerve cords are connected by transverse commissures at right angles in a ladder-like fashion (Reisinger 1925; Bullock and Horridge 1965; Reuter et al. 1998).

According to Lang (1884) and Prudhoe (1985), the polyclad nervous system does not provide information of phylogenetically significant value. However, I found sufficient differences in the CNS that allowed me to distinguish three categories of nervous system arrangements and components. All acotylean polyclads examined fall into Category I, in which well-developed external globuli cell masses are the defining characteristic. The cells are grouped in a cup-shaped fashion from which thin axons extend, forming a stalk that perforates the brain capsule. Similar structures have been found in nemertines (Hanström 1928), annelids including sipunculids (Golding 1992; Åkesson 1958), mollusks (Bullock and Horridge 1965) and especially arthropods, where they are known as "mushrooms bodies" (Schürman 1995; Strausfeld et al. 2006). The arthropod mushrooms bodies are neuropils of thin axons originating from clusters of small basophilic cells. They are part of the CNS and have been implicated in olfaction, learning, and memory (Strausfeld et al. 2006). The external globuli cell masses of all Category I species are similar in composition and position with those in arthropods. Their function in polyclads remains unresolved. If the external globuli cell masses of acotyleans prove to be homologous to the mushrooms bodies of arthropods, then these structures might be an early invention in the evolution of invertebrate nervous systems. However, further studies are needed to clarify the function of these structures and to establish homology.
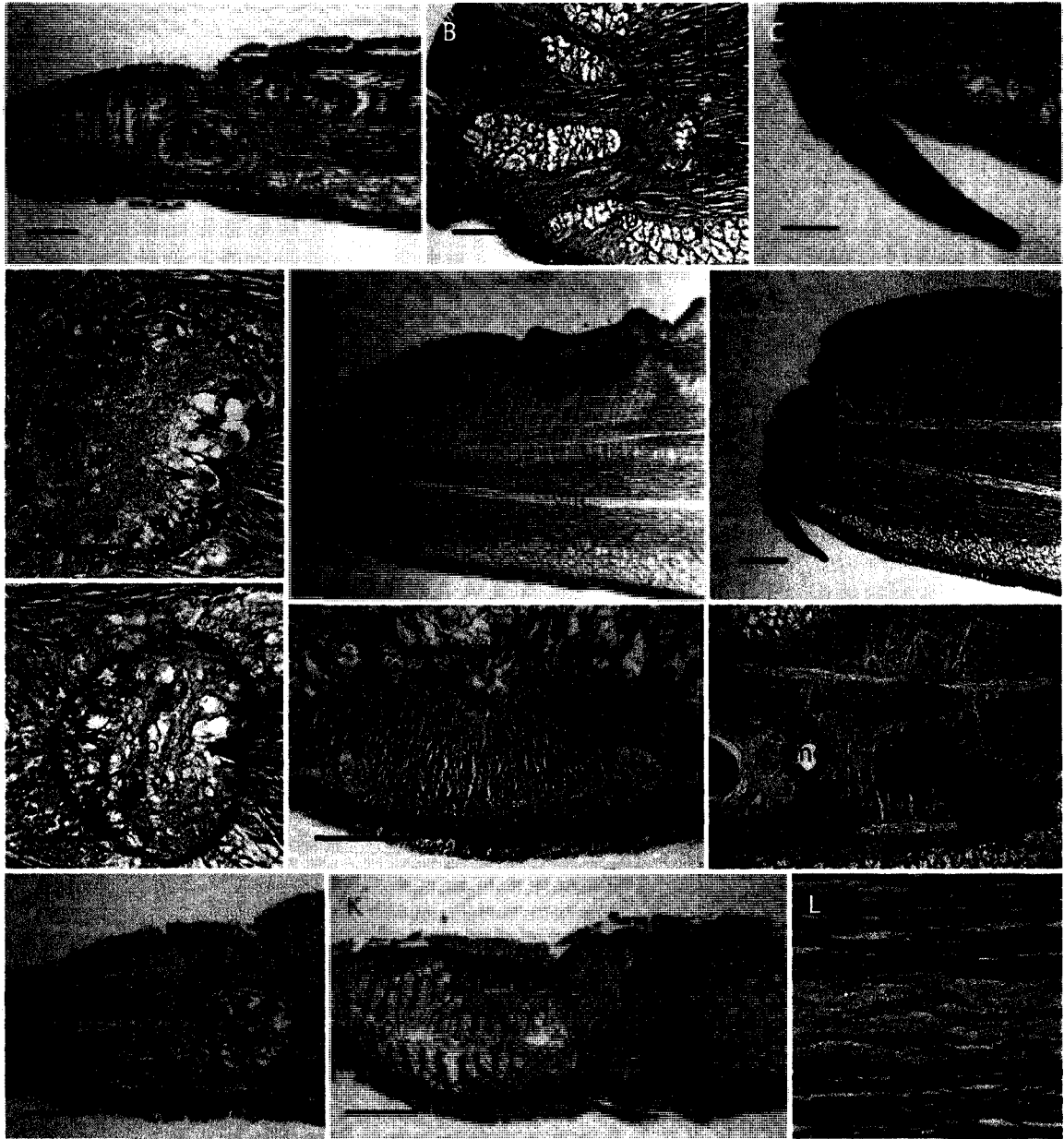
Figure 28. Histological sections of *Boninia divae* (Category III). A. Sagittal section, showing longitudinal musculature surrounding the CNS. Scale bar = 150 μm. B. Longitudinal section through the anterior region, showing the distribution of the ventral nervous branches and immersion of entire CNS into the longitudinal musculature. Scale bar = 150 μm. C. Sagittal section through the left tentacle, showing the arrangement of the tentacular eyes. Scale bar = 150 μm. D. Longitudinal section through the brain. Arrows indicate the Type I cell bodies in the anterior portion of the brain. Scale bar = 75 μm. E. Longitudinal section through the brain. Arrows indicate cells bodies (type III) in the anterior portion of the brain. Notice that the globuli cell masses are formed by cells type II and are poorly defined. Scale bar = 75 μm. F. Anterior sagittal section showing the parallel branches and transverse commissures, forming an orthogonal pattern (portion circled). Scale bar = 250 μm. G. Anterior sagittal section showing dichotomous ramifications (indicated by arrows) anterior to the level of the brain and forming a radial pattern.

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Scale bar = 250 μm. H. Cross section through a ventral commissure. Scale bar = 250 μm. I. Longitudinal section through the reproductive plexus (arrows). Scale bar = 500 μm. J. Sagittal section through the anterior portion of the specimen, showing the dorsal arrangement of some anterior nervous branches. Scale bar = 150 μm. K. Longitudinal section showing the dorsal plexus indicated by arrows. Scale bar = 500 μm. L. Sagittal section through the ventral nerve cord submersed in the longitudinal musclulature. Notice that the nerve cord is surrounded by a thin sheath (arrows). Scale bar = 100 μm. Brain (b); brain capsule (bc); cement glands (cg); eyes (e); female gonopore (fg); globuli cell masses (gcm); longitudinal muscles (lm); male gonopore (mg); nerve cords (nc); pharynx (ph); prostatoids (p); ventral comissures (vcs); ventral nerve cord (vnc)

Although slight differences in globuli cell masses can be distinguished among species of Category I, all acotyleans have external globuli cell masses, which always form mushroom bodies. Although *Pericelis cata* belongs to the Cotylea, it was assigned to this category because its brain contains four external globuli cell masses that form mushrooms bodies. This may be indicative of a close relationship with acotyleans. Other characteristics of Pericelidae linking them to the Acotylea include a centrally located ruffled pharynx, anteriorly directed uteri, and marginal eyes that surround the entire body. On the other hand, the presence of a ventral sucker, pseudotentacles, and uterine vesicles places the family into the Cotylea. A cladistic analysis of Cotylea resolved Pericelidae as a basal clade within the suborder and as sister group to another enigmatic polyclad family, the Boniniidae (Rawlinson and Litvaitis 2008).

All cotyleans examined with the exception of *Pericelis cata*, belong to Category II, which is characterized by poorly developed globuli cell masses. In some cases, such as the Pseudocerotidae, they were completely absent. Furthermore, globuli cell masses of this category never form mushrooms bodies.

Kotikova (1986) considers the CNS of polyclads an orthogon that has been altered by the dorsoventral flattening and spreading out of the body. Features, such as the position and size of the brain, the thickness of the nervous branches and their distribution probably are more related to the shape and position of the pharynx and possibly to different behaviors of the species. Active swimmers (e. g., cotyleans) have

smaller brains and thinner nerve cords, whereas species with mostly cryptic and benthic behaviors (e. g., acotyleans) have larger brains and nerve cords of larger diameters. Although *Pericelis cata* belongs to the Cotylea, it is a species with highly cryptic behavior. In general, cotyleans are characterized by having an anteriorly located pharynx, displacing the brain more to the front of the animal, closer to the anterior margin. In contrast, the pharynx in Acotylea is more centrally located and hence the position of the brain is further removed from the body margin. Finally, in more cryptic species, glomeruli cell masses are better developed possibly indicating a functional need for more complex neural integration.

The particular fleshy consistency and elongated body shape of Boniniidae and their type of locomotion require special modifications of their nervous systems. Species in this group are commonly found under rocks with smooth surfaces in the supralitoral zones. Their movement is best described as "leech-like." Their ventral longitudinal musculature is extremely well developed, occupying almost half of the diameter of the worms. Although their CNS is still placed ventrally, it is not submuscular as in the other rhabditophoran flatworms. Instead, the CNS of Boniniidae is completely immersed in the longitudinal muscles thus forming an intramuscular plexus.

All members of the family Boniniidae have the same overall body structure and main differences separating species are encountered in the male and female reproductive systems (Faubel 1984a; Prudhoe 1985). The unique characteristics of the boninid CNS could thus be considered an autoapomorphy of the family. However, the fact that *Boninia divae* exhibits an orthogonal pattern demonstrates that this configuration has arisen in parallel in different groups as previously suggested by Kotikova (1986, 1991).

The phylogenetic position of Boniniidae is controversial because it shares characteristics of cotyelans and acotyleans. Bock (1923) includes Boniniidae within

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Cotylea because of their marginal tentacles, the arrangements of the eyes, the sucker, and arrangement of uteri. This study further supports such a taxonomic placement because of the poorly defined globuli cell masses.

In summary, the structure and arrangement of the CNS in polyclads is probably related to their body shape and behavior. However, structures such as the external globuli cells masses can provide some information about relationships within the order. Ultrastructural and immunocytological information regarding the CNS of other polyclad species could ultimately prove the usefulness of nervous system characters in phylogenetic reconstructions.

CHAPTER VI

CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

The chapters of this dissertation contribute to a better understanding of the biodiversity, taxonomy and systematics of polyclads. Here, I have shown that polyclads are well represented along the Caribbean coast of Colombia, despite the low number of sites sampled. It is highly likely that an increased number of sampling sites would result in an increase in species.

The newly described species from the deep sea and the continental slope not only increase our knowledge of the biodiversity of these ecosystems but their described associations with bivalves contribute to a further understanding of ecological processes in these types of environments. Because of special requirements for the fixation of polyclads, future studies would be enhanced by close collaborations between flatworm taxonomists and oceanographers to assure proper preservation of deep-sea specimens. Properly processed material would facilitate identifications and result in high quality type material. Furthermore, deep-sea material is now routinely preserved in formalin, which prevents DNA comparisons to other polyclad samples. Proper preservation for DNA studies would greatly advance our knowledge of deep-sea polyclads.

I demonstrated that the central nervous system (CNS) of polyclads shows differences at the light microscopic level that are of systematic value. Future research

approaches should focus on ultrastructural characters, as well as incorporating immunocytochemistry, neuron mapping, and fluorescence microscopy. An examination of the interplay of the nervous and muscular system during embryonic organogenesis and during regeneration using fluorescent markers should provide valuable information on the systematic position of polyclads within the Rhabditophora and within the Bilateria as a whole. Finally, we now have the tools available to understand gene expression during development and regeneration. Using polyclads as models, such studies could provide clues to metazoan evolution in general and may have implications for our understanding of the bilaterian stem species and the diploblast/triploblast transition.

LIST OF REFERENCES

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Åkesson, B. (1958) A study of the nervous system of the Sipunculoideae with some remarks on the development of the two species *Phasoclion strombi* Montagu and *Golfingia minuta* Keferstein. Undersökningar över Öresund 38, 1-249.

Ang, H. P., and Newman, L. J. (1998) Warning colouration in pseudocerotid flatworms (Platyhelminthes, Polycladida). A preliminary study. Hydrobiologia 383, 29-33.

Bàguña, J., Salo, E., Romero, R., Garcia-Fernandez, J., Bueno, J., Muñoz-Marmol, A. M., Bayasca-Ramirez, J. R. and Casali, A. (1994). Regeneration and pattern formation in planarians: cells, molecules, and genes. Zoological Science 11, 781-795.

Bernardo, K., Stone, G. C., Koopowitz H. (1977) Primitive nervous system: habituation in decerebrate flatworms. Journal of Neurobiology 8, 141-150.

Bock, S. (1913). Studien über Polycladen. Zoologiska Bidrag fran Uppsala 2, 29-344.

Bock, S. (1923) *Boninia,* a new polyclad genus from the Pacific. Nova Acta of the Royal Society Uppsala 6, 1-32.

Bolaños, M., Quiroga, S. and Litvaitis, M. K. (2006). A new acotylean flatworm, *Armatoplana colombiana* n. sp. (Platyhelminthes: Polycladida: Stylochoplanidae) from the Caribbean coast of Colombia, South America. Zootaxa 1162, 53-64.

Bolaños, M., Quiroga, S. and Litvaitis, M. K. (2007). Five new species of cotylean flatworms (Platyhelminthes: Polycladida) from the wider Caribbean. Zootaxa 1650, 1-23

Brunner, C. A., Normak, W. R., Zuffa, G. G. and Serra, F. (1999) Deep-sea sedimentary record of the late Wisconsin cataclysmic floods from the Colombia River. Geology 27 (5), 463-466.

Bullock, T. H., Horridge G. A. (1965) Structure and function of the nervous system of invertebrates. W.H. Freeman and Co, San Francisco.

Carté, B. K. (1996) Biomedical potential of marine natural products. Bioscience 46, 271-286.

Cary, S. C., Fisher, C. R. and Felbeck, H. (1988) Mussel growth supported by methane as sole carbon and energy source. Science 240, 78-80.

Child, C. M. (1904a). Studies on regulation. V. The relationship between central nervous system and regeneration in *Leptoplana:* posterior regeneration. Journal of Experimental Zoology 1, 95-133.

Child, C. M. (1904b). Studies on regulation. VI. The relationship between the central nervous system and regeneration in *Leptoplana:* anterior and lateral regeneration. Journal of Experimental Zoology 1, 513-557.

Child, C. M. (1910). The central nervous system as a factor in the regeneration of polyclad Turbellaria. The Biological Bulletin, Woods Hole 19, 333-338

Childress, J. J., Fisher, C. R., Brooks, J. M., Kennicutt, M. C., Bidigare, R. R. and Anderson, A. E. (1986) A methanotrophic marine molluscan (Bivalvia, Mytilidae) symbiosis: Mussels fueld by gas. Science 233, 1306-1308.

Chintala, M. M. and Kennedy, V. S. (1993) Reproduction of *Stylochus ellipticus* (Platyhelminthes: Polycladida) in response to temperature, food, and presence or absence of a partner. Biological Bulletin 185, 373-387.

Christensen, D. J. (1973) Prey preference of *Stylochus ellipticus* in Chesapeake Bay. Proceedings of the National Shellfish Association 63, 35-38.

Cook, C. E., Jiménez, E., Akam, M. and Saló, M. (2004). The Hox gene complement of acoel flatworms, a basal bilaterian clade. Evolution & Development 6 (3), 154-163.

Cordes, E. E., Carney, S. L., Hourdez, S., Carney, R. S., Brooks, J. M. and Fisher, C. R. (2007) Cold seeps of the deep Gulf of Mexico: Community structure and biogeographic comparisons to Atlantic equatorial belt seep communities. Deep-Sea Research I 54, 637-63.

Deutsch, J. S. (2008) Do acoels climb up the "Scale of Beings"? Evolution & Development 10 (2), 135-140

Dunn, C. W., Hejnol, A., Matus, D. Q., Pang, K., Browne, W. E., Smith, S. A., Seaver, E., Rouse, G. W., Obst, M., Edgecombe, G. D., Sørensen, M. V., Haddock, S. H. D., Schmidt-Rhaesa, A., Okusu, A., Kristensen R. M., Wheeler, W. C., Martindale, M. Q. and Giribet, G. (2008). Broad phylogenomic sampling improves resolution of the animal tree of life. Nature 452, 745-750.

Ehlers, U. (1986). Comments on a phylogenetic system of the Platyhelminthes. Hydrobiologia 132, 1-12.

Faubel, A. (1983) The Polycladida, Turbellaria. Proposal and establishment of a new system. Part I. The Acotylea. Mitteilungen des hamburgischen zoologischen Museums und Instituts 80, 17–121.

Faubel, A. (1984a) The Polycladida, Turbellaria. Proposal and establishment of a new system. Part II. The Cotylea. Mitteilungen des hamburgischen zoologischen Museums und Instituts 81, 189–259.

Faubel, A. (1984b) On the geographical occurrence of pelagic polyclad turbellarians. Cahiers de Biologie Marine 25, 153-168.

Galleni, L., Tongiorgi, P., Ferroro, E. and Salghetti, U. (1980) *Stylochus mediterraneus* (Turbellaria: Polycladida), predator on the mussel Mytilus galloprovincialis. Marine Biology 55, 317-326.

Golding, D.W. (1992) Polychaeta: nervous system. In: Harrison F.W., Gardiner S.L. (Eds), Microscopic Anatomy of Invertebrates. Vol. 7. Annelida. Wiley and Liss, New York, pp. 153-179.

Goggin, C. L. and Newman, L. J. (1996). Use of molecular data to discriminate pseudocerotid turbellarians. Journal of Helminthology 70 (2), 123-6.

Hadenfeldt, D. (1929) Das Nervensystem von Stylochoplana maculata und Notoplana atomata. Zeitschrift für wissenschaftliche. Zoologie 133, 586-638.

Hanström, B. (1928) Vergleichende Anatomie des Nervensystems der wirbellosen Tiere unter Berücksichtigung seiner Funktion. Springer Verlag, Berlin.

Herring, P. (2002) The Biology of the Deep Ocean. Biology of Habitats. Oxford University Press, 314 pp.

Hooge, M. and Newman L. J., Turbellarians of the Gulf of Mexico. In Gulf of Mexico Origins, Waters, and Biota - Biodiversity, (ed. D. L. Felder). Texas A & M University Press, in press.

Hyman, L. H. (1940) The polyclad flatworms of the Atlantic coast of the United States and Canada. Proceedings of the United States National Museum 89, 449-495.

Hyman L. H. (1951) The Invertebrates: Vol. II. Platyhelminthes and Rhynchocoela; the acelomate Bilateria. McGraw-Hill, New York, 572pp

Hyman, L. H. (1954a) The polyclad genus Pseudoceros, with special reference to the Indo-Pacific region. Pacific Science 8, 219-225.

Hyman, L. H. (1954b). Free-living flatworms (Turbellaria) of the Gulf of Mexico. Fisheries Bulletin of the Fish and Wildlife Service 55, 301-302.

Hyman, L. H. (1955a) Some polyclads from the West Indies and Florida. Proceedings of the United States National Museum 104, 115-150.

Hyman, L. H. (1955b) A further study of the polyclad flatworms of the West Indian region. Bulletin of Marine Science of the Gulf and Caribbean 5, 259-268.

Hyman, L. H. (1959a) A further study of Micronesian polyclad flatworms. Proceedings of the United States National Museum 108, 543-597.

Hyman, L. H. (1959b) Some Australian polyclads. Records of the Australian Museum 25, 1-17.

Jennings, K. A., and Newman, L. J. (1996a) Two new stylochids (Plathyhelminthes, Polycladida) from the Great Barrier Reef, Australia. The Raffles Bulletin of Zoology 44, 135-142.

Jennings, K. A., and Newman, L. J. (1996b) Four new stylochid flatworms associated with oysters from southeast Queensland, Australia. The Raffles Bulletin of Zoology 44, 493-508.

Jeon, J. K., Miyawa, K., Noguchi, T., Narita, H., Matsubara, S., Nara, M., Ito, K. and Hashimoto, K. (1987). Distribution of tetrodotoxin in the tissue of the flatworm Planocera multitentaculata (Platyhelminthes). Toxicon 25, 975-980.

Karling, T. G. (1967). Zur Frage von dem systematischen Wert der Kategorien Archoophora und Neoophora (Turbellaria). Comm Biol Soc Fenn 30, 1-11.

Karling, T. G. (1974). On the anatomy and affinities of the turbellarian orders. In: Biology of the Turbellaria. Riser NW, Morse MP (eds), pp 1-16. McGraw-Hill, New York.

Kennicutt, M. C., Brooks, J. M., Bidigare, R. R. and Denoux, G. J. (1988) Gulf of Mexico hydrocarbon seep communities. I. Regional distribution of hydrocarbon seepage and associated fauna. Deep-Sea Research A 35, 1639-1651.

Kennicutt, M. C., Brooks, J. M., Bidigare, R. R., Fay, R. R., Wade, T. L. and MacDonald, D. J. (1985) Vent-type taxa in a hydrocarbon seep region on the Louisiana slope. Nature 317, 351-353.

Koopowitz, H. (1973) Primitive nervous system. A sensory nerve net in the polyclad flatworm Notoplana acticola. Biological Bulletin 145, 352-359.

Koopowitz, H. (1974) Some aspects of the physiology and organization of the nerve plexus in polyclad flatworms. In: Riser, N.W., Morse, M.P. (Ed), Biology of the Turbellaria. McGraw-Hill, New York, pp. 198-212

Koopowitz, H. (1986) On the evolution of the central nervous systems: implications from polyclad turbellarian neurobiology. Hydrobiologia 132, 79-87.

Kotikova, E.A. (1986) Comparative characterization of the nervous system of the Turbellaria. Hydrobiologia 132, 89-92.

Kotikova, E.A. (1991) The orthogon of the Platyhelminthes and main trends of its evolution. Proceedings of the Zoological Institute, St. Petersburg 241, 88-111.

Kubanek, J., Williams, D. E., deSilva, E. D., Allen, T. and Andersen, R. J. (1995) Cytotoxic alkaloids from the flatworm Prostheceraeus vittatus and its tunicate prey Clavelina lepadiformis. Tetrahedron Letters 36, 6189-6192

Laidlaw, F. F. (1902). The marine Turbellaria, with an account of the anatomy of some of the species. Fauna Georg Maldvine Laccadive Archipelagoes 1, 282-312.

Lang, A. (1879) Untersuchungen zur vergleichenden Anatomie und Histologie des Nervensystems der Plathelminthen. I. Das Nevensystem der marinen Dendrocoelen. Mittheilungen aus der Zoologischen Station, Neapel. 1, 459-488.

Lang, A. (1884) Die Polycladen (Seeplanarien) des Golfes von Neapel und der angrenzenden Meeresabschnitte. Eine Monographie. Fauna und Flora des Golfes von Neapel 11. 688 pp. Leipzig.

Levetzow, K. G. (1939). Die Regeneration der Poycladen Turbellarien. Wilhelm-Roux Arch EntwMech Org 139, 780-818.

Litvaitis, M. K. and Newman, L. J. (2001) A molecular framework for the phylogeny of the Pseudocerotidae (Platyhelminthes, Polycladida). Hydrobiologia 444, 177–182.

Litvaitis, M. K. and Rohde, K. (1999). A molecular test of platyhelminth phylogeny: inferences from partial 28rDNA sequences. Invertebrate Biology 118, 42-56.

MacAvoy, S. E , Macko, S.A, Carney, R.S. (2003) Links between chemosynthetic production and mobile predators on the Louisiana continental slope: Stable carbon isotopes of specific fatty acids. Chemical Geology 201, 229-239.

MacDonald, I. R., Reilly, J. F., Guinasso, N. L., Brooks, J. M., Carney, R. S., Bryant, W. A. and Bright, T. J. (1990) Chemosynthetic mussels at a brine-filled pockmark in the northern Gulf of Mexico. Science 248, 1096-1099.

Marcus, E. (1952) Turbellaria Brasileiros (10) Boletin da facultade de filosofia ciencias y letras de Sao Paulo. Zoologia No 17, 5-188.

Marcus, E. (1954) Reports of the Lund University Chile Expedition 1948-49. 11. Turbellaria. Lunds Universitets Arsskrift. N. F. 49 (13), 1-115.

Marcus, E. (1960) Turbellaria from Curaçao. Studies on the Fauna of Curaçao and other Caribbean Islands 44, 41-51.

Marcus, Ev., and Marcus, E. (1968) Polycladida from Curaçao and faunistically related regions. Studies on the Fauna of Curaçao and other Caribbean Islands 26, 1-133.

Minichev, Y. S., Pugovkin, A. P. (1979) Nervous system of the polyclad flatworm *Notoplana atomata* (O.F. Müller). Les Cahiers de Biologie Marine 20, 181-188.

Miyazawa, K., Jeon, J. K., Noguchi, T., Ito, K and Hashimoto, K. (1987). Distribution of tetrodotoxin in the tissues of the flatworm *Planocera multitentaculata* (Platyhelminthes). Toxicon 25, 975-980.

Murina, G. V., Grintsov, V., and Solonchenko, A. (1995) *Stylochus tauricus,* a predator of the barnacle *Balanus improvisus* in the Black Sea. Hydrobiologia 305, 101-104.

Newell, R. I. E., Alspach, G. S., Kennedy, V. S. and Jacobs, D. (2000) Mortality of newly metamorphosed eastern oysters *(Crassostrea virginica)* in mesohaline Chesapeake Bay. Marine Biology 136, 665-676.

Newman, L. J., and Cannon, L. R. G. (1994a) Biodiversity of Australian polyclad flatworms. Memoirs of the Queensland Museum 36, 159-163.

Newman, L. J., and Cannon, L. R. G. (1994b) *Pseudoceros* and *Pseudobiceros* (Polycladida, Pseudocerotidae) from Eastern Australia and Papua New Guinea. Memoirs of the Queensland Museum 37, 205-266.

Newman, L. J., and Cannon, L. R. G. (1995) The importance of the fixation of colour, pattern and form in tropical Pseudocerotidae (Platyhelminthes, Polycladida). Hydrobiologia 305, 141-143.

Newman, L. J., and Cannon, L. R. G. (1996a) New genera of pseudocerotid flatworms (Platyhelminthes, Polycladida) from Australian and Papua New Guinean coral reefs. Journal of Natural History 30, 1425- 1441.

Newman, L. J., and Cannon, L. R. G. (1996b) *Bulaceros,* new genus and *Tytthosoceros,* new genus (Platyhelminthes, Polycladida, Pseudocerotidae) from the Great Barrier Reef, Australia and Papua New Guinea. The Raffles Bulletin of Zoology 44, 479-492.

Newman, L. J., and Cannon, L. R. G. (1997) Nine new *Pseudobiceros* (Platyhelminthes, Polycladida, Pseudocerotidae) from the Indo-Pacific. The Raffles Bulletin of Zoology 45, 341-368.

Newman, L. J., and Cannon, L. R. G. (1998) New *Pseudoceros* (Platyhelminthes, Polycladida, Pseudocerotidae) from the Indo-Pacific. The Raffles Bulletin of Zoology 46, 293-323.

Newman, L. J., and Cannon, L. R. G. (2000) A new genus of euryleptid flatworm (Platyhelminthes, Polycladida, Euryleptidae) from the Indo-Pacific. Journal of Natural History 34, 191-205.

Newman, L. J., and Cannon, L. R. G. (2002) The genus *Cycloporus* (Platyhelminthes, Polycladida) from Australasian waters. The Raffles Bulletin of Zoology 50, 287-299.

Newman, L. J., and Cannon, L. R. G. (2003) Marine Flatworms: the world of polyclad flatworms. CSIRO Publishing, Melbourne, Victoria, 112 pp.

Newman, L. J., Cannon, L. R. G. and Govan, H. (1993) *Stylochus (Imogene) matatasi* n. sp. (Platyhelminthes, Polycladida): Pest of cultured giant clams and pearl oysters from Solomon Islands. Hydrobiologia 257, 185-189.

Newman, L. J., Paulay, G., and Ritson-Williams, R. (2003). Checklist of polyclad flatworms (Platyhelminthes) from Micronesian coral reefs. Micronesica 35-36, 189-199.

O'Connor, W. A. and Newman, L. J. (2001) Halotolerance of the oyster predator, *Imogine mcgrathi,* a stylochid flatworm from Port Stephens, New South Wales, Australia. Hydrobiologia 459, 157-163.

Olmsted, J. M. D. (1922). The role of the nervous system in the regeneration of polyclad Turbellaria. Journal of Experimental Zoology 36, 48-56.

Pailleret, M., Haga, T., Petit, P., Prive-Gill, C., Saedlou, N., Gaill, F. and Zbinden, M. (2007) Sunken wood from the Vanuatu Islands: identification of wood substrates and preliminary description of associated fauna. Marine Ecology 28, 233- 241.

Palombi, A. (1924) Policladi pelagici. Raccolte planktoniche fatte dalla R. N. "Liguria" nel viaggio di circonnavigazione del 1903-05. Pubblicazioni Recerchi del Istituto degli Studi Superiori Firenze 3, 1-28.

Paull, C. K., Hecker, B., Commeau, R., Freeman-Lynde, R. P., Neumann, C., Corso, W. P., Golubic, S., Hook, J. E., Sikes, E. and Curray, J.(1984) Biological communities at the Florida Escarpment resemble hydrothermal vent taxa. Science 226, 965-967

Pearse, A. S., and Wharton, G. W. (1938) The oyster "leech", *Stylochus inimicus* Palombi, associated with oysters on the coasts of Florida. Ecological Monographs 8, 605-655.

Philippe, H., Brinkmann, H., Martinez, P., Riutort, M. and Baguñà, J. (2007) Acoel flatworms are not Platyhelminthes: Evidence from phylogenomics. PLoS ONE 2(8): e717. doi:10.1371/journal.pone.0000717

Prensnell, J.K., Schreibman, M.P. (1997) Humason's Animal Tissue Techniques. Fifth ed. The John Hopkins University Press, Baltimore, 572 pp.

Provenzano, A. J. (1961) Effects of the flatworm *Stylochus ellipticus* (Girard) on oyster spat in two salt water ponds in Massachusetts. Proceedings of the National Shellfish Association 50, 83-88.

Prudhoe, S. (1944) On some polyclad turbellarians from the Cayman Islands. Annales and Magazine of Natural History 11, 322-334.

Prudhoe, S. (1985) A Monograph on Polyclad Turbellaria. Oxford University Press: Oxford, 259 pp.

Prudhoe, S. (1989) Polyclad turbellarians recorded from African waters. Bulletin of the British Museum of Natural History 55, 47-96.

Quiroga, S., Bolaños, M. and Litvaitis, M. K. (2006) First description of deep-sea polyclad flatworms from the North Pacific: *Anocellidus* n. gen. *profundus* n. sp. (Anocellidae, n. fam.) and *Oligocladus voightae* n. sp. (Euryleptidae). Zootaxa 1317, 1-19.

Quiroga, S., Bolaños, M. and Litvaitis, M. K. (2008). Two new species of flatworms (Platyhelminthes: Polycladida) from the continental slope of the Gulf of Mexico. Journal of the Marine Biological Association of the United Kingdom (In press.)

Rawlinson, K. A., Litvaitis, M. K. (2008) Cotylea (Polycladida): a cladistic analysis of morphology. Invertebrate Biology 127, 121-138.

Ritson-Williams, R., Yotsu-Yamashita, M. and Paul, V. J. (2006) Ecological functions of tetrodotoxin in a deadly polyclad flatworm. Proceedings of the National Academy of Sciences 103, 3176-3179.

Reisinger, E. (1925) Untersuchungen am Nervensystem der *Bothrioplana semperi* Braun. Zeitschrift für Morphologie und Oekologie der Tiere 5, 119-149.

Reuter, M., Mäntylä, K., Gustafsson, M.K.S. (1998) Organization of the orthogon – main and minor nerve cords. Hydrobiologia 383, 175-182.

Ruiz-Trillo, I., Riutort, M., Littlewood, D.T.J., Herniou, E.A. and Baguñà, J. (1999) Acoel flatworms: earliest extant bilaterian Metazoans, not members of Platyhleminthes. Science 238, 1919-1923

Salvador, A. (1987) Late Triassic – Jurassic paleogeography and origin of the Gulf of Mexico. American Association of Petroleum Geologists Bulletin 71, 419-451.

Salo, E., Tauler, J., Jimenez, E., Bayascas, J. R., Gonzalez-Linares, J., Garcia-Fernandez, J. and Bàguña, J. (2001). Hox and Parahox genes in flatworms: Characterization and expression. American Zoologist 41, 652-663.

Schupp, P., Eder, C., Proksch, P., Wray, V., Schneider, B., Herderich, M. and Paul, V. (1999). Staurosporine derivatives from the ascidian *Eudistoma toealensis* and its predatory flatworm *Pseudoceros sp.* Journal of Natural Products 62, 959-962.

Schürman, F. W. (1995) Common and special features of the nervous system of Onychophora: A Comparison with Arthropoda, Annelida and some other invertebrates. In: Breidback, O., Kutsch, W. (Ed), The Nervous System of Invertebrates: An Evolutionary and Comparative Approach. Birkhäuser, Switzerland, pp. 139-157.

Strausfeld, N. J., Strausfeld, C. M., Loesel, R., Rowell, D., Stowe, S. (2006) Arthropod phylogeny: onychophoran brain organization suggest an archaic relationship with a chelicerate stem lineage. Proceedings of the Royal Society B 273, 1857-1866.

Turner, R. D. (1978) Wood, mollusks, and deep-sea food chains. Bulletin of the American Malacological Union for 1977, 13-19.

Van Dover, C. L. (2000) The ecology of deep-sea hydrothermal vents. Princeton University Press, 424 pp.

Van Dover C. L., Humphris, S. E., Fornari, D.M., Cavanaugh, C. M., Collier, R., Goffredi, S. K., Hashimoto, K., Lilley, M. D., Reysenbach, A. L., Shank, T. M., VonDamm, K. L., Banta, A., Gallant, R. M., Götz, D., Green, D., Hall, J., Harmer, T. L., Hurtado, L. A., Johnson, P., McKiness, Z. P., Meredith, C., Olson, E., Pan, I. L., Turnipseed, M., Won, Y., Young, C. R. and Vrijenhoek, R. C. (2001) Biogeography and ecological setting of Indian Ocean hydrothermal vents. Science 294, 818-823.

Verrill, A. E. (1892) Marine Planarians of New England. Transactions of the Connecticut Academy of Arts and Sciences 8, 60-520.

Villalba, A., Mourelle, S. G., Carballal, M. J. and Lopez, C. (1997) Symbionts and diseases of farmed mussels *Mytilus galloprovincialis* throughout the culture process in the Rias of Galicia (NW Spain). Diseases of Aquatic Organisms 31, 127-139.

Voight, J. R. (2005) First report of the enigmatic echinoderm *Xyloplax* from the North Pacific. Biological Bulletin 208, 77-80.

Voight, J. R. (2007) Experimental deep-sea deployments reveal diverse Northeast Pacific wood-boring bivalves of Xylophagainae (Myoida: Pholadidae). Journal of Molluscan Studies 73, 377-392.

Webster, J. R. and Medford, R. Z. (1961) Flatworm distribution and associated oyster mortality in Chesapeake Bay. Proceedings of the National Shellfish Association 50, 89-96.

Young, C. M., Sewell, M. A. and Rice, M. E. (2006). Atlas of Marine Invertebrate Larvae. Academic Press, London. 626 pp

APPENDICES

APPENDIX A

Fixation and Embedding of Polyclad Flatworms

Fixation and preservation: Coax the specimen onto a piece of filter paper using a fine paint brush and place the filter paper on frozen fixative; add a thin layer of cold fixative. Use a small paintbrush to keep the animal flat as it is being fixed. Keep worms at 4°C overnight, then transfer and store in 70 % ethanol. This method ensures the animals are fixed flat.

Fixatives: choice of fixative depends on final purpose

1. 10% buffered formalin (used for traditional histology and whole mounts)

2. Trumps Fixative (used for traditional histology, whole mounts and TEM)

   1% glutaraldehyde and 4% formaldehyde in 0.1 M phosphate buffer, pH 7.2

Embedding:

1. 70% ETOH for 30 minutes.

2. 90% ETOH for 30 minutes.

3. 95% ETOH for 30 minutes.

4. 100% ETOH. Two times for 30 minutes each.

5. ETOH and Histoclear 1:1, for 30 minutes.

6. 100% Histoclear. Two times for 30 minutes each.

7. Histoclear and melted Paraplast in a 1:1 ratio.

8. Incubate for 30 munites at 58ºC.

9. Change Paraplast two times for 30 minutes each.

10. Embed tissue in freshly melted Paraplast.

Preparation of Whole Mounts

1. Using the entire worm, follow the procedure for embedding to step 6.

2. If the worm is too transparent, stain with Eosin for 1 min and rinse with distilled water

3. Mount the whole worm in Permount

4. Allow to dry for several days

Sectioning and Staining

Section at about 5-7 μm

Float section on warm water to flatten out

Pick them up on positively charge slide and allow to dry. Stain the sections as follows:

1. Histoclear. Two times for 10 munites.

2. ETOH 100%. Two times for 5 minutes.

3. ETOH 95%. Two times for 5 minutes.

4. ETOH 70% for 5 minutes.

5. Distiled water for 5 minutes.

6. Hematoxilin for 6 minutes.

7. Rinse in running tap water; make sure not to rinse section off slides.

8. Acid alcohol for 1 to 5 seconds.

9. Rinse in running tap water.

10. Eosin for 15 seconds.

11. ETOH 95%.Two times for 5 minutes.

12. ETOH 100%. Two times for 5 minutes.

13. Histoclear. Two times for 5 minutes.

14. Allow to dry and mount with Permount.

Protocol for Milligan Trichrome Stain

1. Deparaffinize with Histoclear. Three times 5 minutes each.

2. Transfer slides to absolute alcohol. Two times 5 minutes each.

3. Transfer slides to 95% alcohol. Two times 5 minutes each.

4. Mordant in potassium dichromate-hydrochloric acid solution for 5 minutes.

5. Rinse with distilled water.

6. Stain with acid fuchsin for 7 minutes.

7. Rinse with distilled water.

8. Fix the stain in phosphomolybdic acid solution for 4 minutes.

9. Stain with Orange G for 8 minutes.

10. Rinse in distilled water.

11. Treat with 1% aqueous acid for 2 minutes.

12. Stain in Fast Green for 8 minutes.

13. Treat with 1% acetic acid for 3 minutes.

14. Rinse in 95% alcohol. Two times 5 minutes each.

15. Transfer into absolute alcohol. Two times for 5 minutes.

16. Finish dehydration with two changes of Histoclear, 5 minutes each.

17. Clear

18. Dry overnight.

19. Mount in Permount.

Working Solutions

Mordant

Solution A

Potassium dichromate 3.0 g

Distilled water 100.0 ml

Solution B

Hydrochloric acid, concentrated 10.0 ml

95% alcohol 100 ml

Mix 3 parts A with 1 part B; use within 4 hours

Acid fuchsin

Acid fuchsin 0.1 g

Distilled water 100.0 ml

Phosphomolybdic acid

Phosphomolybdic acid 2.0 g

Distilled water 200.0 ml

Orange G

Orange G 2.0 g

1% phosphomolybdic acid 100 ml

Fast Green

Stock solution

Fast Green FCF 10.0 g

2% acetic acid (2ml/98ml destilled water) 100.0 ml

Working solution

Fast Green stock solution 10.0 ml

Distilled water 90.0 ml