

**Family Macrovalvitrematidae Yamaguti, 1963 (Monogenea: Heteronchoinea: Oligonchoinea): Study and Descriptions of the Puerto Rican Fauna, Phylogeny and Revised Classification, and Coevolutionary Events Resulting from the Reconstruction of the Host Phylogeny Based on Parasite Data**

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## Abstract

The Class Monogenea van Beneden, 1858 comprises a diverse group of platyhelminthes parasitic primarily on the external surfaces and gills of marine and freshwater fishes. The family **Macrovalvitrematidae** Yamaguti, 1963 is a small heteronchoinean family with distinctive anterior end and opisthaptor. This work includes the study of the macrovalvitrematid fauna on fishes of Puerto Rico, the analysis of characters, the phylogeny and a revised classification of the family Macrovalvitrematidae. Also the reconstruction of the host phylogeny based in the parasite data with notes in possible coevolutionary events and host specificity are discussed. Fishes of the families Sciaenidae, Haemulidae, Sparidae were collected from the west coast of Puerto Rico (Mayagüez and Cabo Rojo) and examined for macrovalvitrematid monogeneans. Also few specimens of the sciaenid *Micropogonias furnieri* were collected from the northeast coast of Puerto Rico (Loíza). The results of this study includes one new species of the genus *Pseudotagia* from the haemulid *Pomadasys corvinaeformis*, representing new host record; one new genus and species from the sparid *Archosargus rhomboidalis* establishing also new locality record; and two new localities records for *Macrovalvitrema argentinensis* comb. nov. and *Neopteriotrematoides avaginata*. Two new combinations are proposed in this study: *Macrovalvitrema argentinensis* (synonym: *Neomacrovalvitrema argentinensis*) and *Nicolasia cortesi* (synonym: *Pseudohargisia cortesi*). The phylogenetic analysis of 42 characters using parsimony results in completely resolved tree (85 steps and 84% of consistency index) suggesting the monophyly of the family. The tree resulting from Bayesian analysis also supports the monophyly of the group with 93% of credibility value. Both analyses also suggests the diclidophorids of the subfamily Diclidophorinae

(*Mamaevicotyle*) as the sister group of the Macrovalvitrematidae resulting in the paraphyly of the Family Diclidophoridae. The host phylogeny suggests a close relation between the perciform families Sciaenidae, Lutjanidae, Sparidae, Haemulidae and Serranidae. The cladograms also indicates the polyphyly of the Percomorpha with the position of the pleuronectiform (flat fish) as sister group of the families Sparidae, Lutjanidae and Sciaenidae. The position of *C. sordidus* suggests a host-switching event associated with the origin of *Papillopseudotagia hubbsi* on *C. sordidus*. The basal position of the Haemulidae is congruent with the position of *Papillopseudotagia* suggesting co-speciation. The high prevalence and mean intensity of *Pseudotagia* on the haemulid *Pomadasys* support the strong relation between this macrovalvitrematid genus and the family Haemulidae. The co-occurrence of the subfamilies Macrovalvitrematinae and Pterinotrematoidinae among numerous sciaenids and the lutjanids indicates that both have coevolved together with the host.

## Resumen

La clase Monogenea van Beneden, 1858 está compuesta por un grupo diverso de platelmintos parasíticos encontrados mayormente en las superficies externas y en las agallas de peces marinos y de agua dulce. La familia **Macrovalvitrematidae** Yamaguti, 1963 es una pequeña familia de heteronchoineos que se distinguen por la forma de la parte anterior del cuerpo (area oral) y su opistaptor. Este trabajo incluye el estudio de la fauna de macrovalvitremátidos de peces de Puerto Rico, el análisis de sus caracteres, la filogenia y clasificación revisada de la familia Macrovalvitrematidae. La reconstrucción de la filogenia de los hospederos sobre la base de datos o información de los parásitos (macrovalvitrematidos) con notas sobre los posibles eventos coevolucionarios entre estos y sobre la especificidad de hospedero que aparentan tener estos monogeneos son discutidos. Peces de las familias Sciaenidae, Haemulidae, Sparidae fueron colectados de las costa oeste de Puerto Rico (Mayagüez and Cabo Rojo) y examinados con la intención de encontrar monogeneos pertenecientes a la familia Macrovalvitrematidae. En adición algunos especímenes del sciaénido *Micropogonias furnieri* se colectaron de la costa noreste de Puerto Rico (Loíza). Los resultados de este estudio incluyen el reporte de una nueva especie del género *Pseudotagia* del haemulido *Pomadasys corvinaeformis*; el reporte de un nuevo género y especie en *Archosargus rhomboidalis* (Sparidae) y dos nuevos registros de localidad para *Macrovalvitrema argentinensis* comb. nov. y *Neopteriotrematoides avaginata*. En este estudio también se proponen dos nuevas combinaciones: *Macrovalvitrema argentinensis* (sinónimo: *Neomacrovalvitrema argentinensis*) y *Nicolasia cortesi* (sinónimo: *Pseudohargisia cortesi*). El análisis filogenético de 42 caracteres usando parsimonia resulta en un cladograma completamente



resuelto (con 85 pasos y un índice de consistencia de 84%) que sugiere la monofilia de la familia Macrovalvitrematidae. El análisis Bayesiano de los mismos 42 caracteres resulta en un cladograma que también apoya la monofilia del grupo con un valor de credibilidad de 93%. Ambos análisis también sugieren la parafilia de la familia Diclidophoridae sugiriendo que *Mamaevicotyle* (Diclidophorinae) es el grupo hermano de la familia Macrovalvitrematidae. La reconstrucción de la filogenia de hospederos sugiere una relación cercana entre las familias del orden Perciformes Sciaenidae, Lutjanidae, Sparidae, Haemulidae and Serranidae. Esta también indica el estatus polifilético de la Serie Percomorpha con la posición de *Citarrichthys sordidus* (Pleuronectiformes: Paralichthyidae) como grupo hermano de las familias Sparidae, Lutjanidae y Sciaenidae. La posición de este sugiere un evento de cambio de hospedero “host-switching” asociado con el origen de *Papillopseudotagia hubbsi* en *C. sordidus*. La posición basal de la familia Haemulidae es congruente con la posición de *Papillopseudotagia* sugiriendo un evento de co-especiación. Los valores altos de prevalencia y de intensidad promedio de *Pseudotagia* en el haémulido *Pomadasys* también apoya la relación estrecha entre este género de macrovalvitrematido y la familia Haemulidae. La co-ocurrencia de las subfamilias Macrovalvitrematinae y Pterinotrematoidinae en numerosos sciaénidos y en una especie de lutjanido indica que ambos han coevolucionado con su hospedero.

## **Dedication**

The effort and sacrifice that represented this work I dedicated to my best partner, my husband Carlos for his help, trust, support and my daily motivation; to my two beautiful and beloved kids Sara Carolina and Gustavo Xavier, which were born and grew during this hard learning process, and were my inspiration; and to my parents for always believe in me.

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## **Chapter I: Introduction**

Monogenea is a class of parasitic platyhelminthes that have been characterized for their host specificity and their simple, direct life cycle that includes only three stages: adult, egg and larva (oncomiracidium). The monogeneans are mainly parasites of marine and freshwater fishes, but parasitize amphibians (infraclass Polystomatoinea) and have been reported from other aquatic animals such as the hippopotamuses and turtles. Poulin (1992) considered the monogeneans good candidates for investigating the evolution of parasite specialization. The literature also suggests that Monogenea is an excellent group to study diversification and diversity because they are considered very host specific, their phylogeny is well resolved at least to family level and they are diverse in number and morphology (Poulin, 2002). The monogeneans have been considered for many authors as one of the most host specific parasites. Whittington (2000) suggests that the monogeneans are among the most host-specific of parasites in general and may be the most host-specific of all fish parasites.

The phylogenetic status of the Class Monogenea has been one of controversy with competing published hypotheses suggesting either polyphyly or monophyly. Monophyly has been suggested in analyses based on morphology (Ehlers, 1984; Brooks, 1989; and Boeger and Kritsky, 1993, 2001) and rejected with molecular analyses (Mollaret et al., 1997) and questioned by studies of sperm structure (Justine, 1991, 1993 and 2001). The Monogenea are split into two large subclasses Polyonchoinea and the Heteronchoinea,

and the monophyly of these two subclasses have been consistently suggested as monophyletic.

The body of monogeneans has three main sections: the anterior end, the body proper and the opisthaptor, but the characteristics or the character states of these three areas vary considerably between the two subclasses. The Polyonchoinea are characterized by the presence of cephalic glands (that aid in suction), eyes spots and relatively large pharynx in the anterior end while the Heteronchoinea do not present the cephalic glands or the eyes spots, but instead have a pair of oral suckers. The genitalia as well as the armature of the opisthaptor also differ considerably between these two subclasses.

The family **Macrovalvitrematidae** Yamaguti, 1963 is a small heteronchoinean family with distinctive anterior end and opisthaptor. Their anterior end is characterized by what Caballero and Bravo-Hollis (1955) and later Yamaguti (1963) named as “goblet-shaped”, and their opisthaptor has eight (four pairs) of clamps that are longer than wide and shaped like a guitar, miter, firetong or raquet. The copulatory organ in most of the macrovalvitrematid species has a genital corona of spines arranged radially similar to those observed in the Family Diclidophoridae. However, the copulatory organ of three species of macrovalvitrematid also is armed with several spines but not in a radial arrangement.

The family Macrovalvitrematidae has not been of high interest for the parasitology community with the exception of Bravo-Hollis, 1982 who divided the reported genera

into two subfamilies (Macrovalvitrematinae and Pterinotrematoidinae) based on the morphology of the opisthaptor and the male genitalia. At that time this is a small family with only eight genera and nine species of which only one genus was not monotypic. Posteriorly Payne (1987) adds one new genus and species to each subfamily. The genera and species included in the family previous to this study were *Macrovalvitrema sinaloense* Caballero y C. and Bravo-Hollis, 1955; *Pterinotrematoides mexicanum* Caballero y C. and Bravo-Hollis, 1955; *Macrovalvitrematoides micropogoni* (Pearse, 1949); *Hargisia bairdiella* (Hargis, 1956), *Pseudotagia cupida* (Hargis, 1956), *Pseudotagia clathratus* Crane, 1972; *Neomacrovalvitrema argentinensis* Suriano, 1975, *Neopterinotrematoides avaginata* Suriano, 1975; *Nicolasia canosorum* Suriano, 1975; *Pseudohargisia cortesi* Payne, 1987; *Papillopseudotagia hubbsi* Payne, 1987 and *Pseudotagia rubri* Luque et al., 1993.

### **Host and Geographical Distribution**

Previous to this study the macrovalvitrematids were reported from the pacific coast of Mexico, California and Perú; the Gulf of Mexico, Virginia and North Carolina in the eastern coast of United States (western north Atlantic); from Argentina and Brazil in the south Atlantic; and from the Gulf of Cariaco and Isla Margarita in Venezuela (Caribbean Sea). The fish families reported as host of the macrovalvitrematids include the Sciaenidae (*Micropogonias*, *Umbrina*, *Ophioscion* and *Bairdiella*), Haemulidae (*Orthopristis* and *Haemulon*), Serranidae (*Paralabrax* and *Diplectrum*), Lutjanidae (*Lutjanus*), Sparidae (*Archosargus*) and Paralichthyidae with noticeable preferences for the Sciaenidae and Haemulidae.

The genus *Micropogonias* appears to be the preferred genus of the sciaenids for the macrovalvitrematids or perhaps this is an artifact of study bias as *Micropogonias* is an important food fish and may be more frequently and better examined.

Macrovalvitrematids have been reported from *Micropogonias ectenes*, *M. megalops* from the Pacific; *M. furnieri* from the south Atlantic including Argentina and Brazil, and the Caribbean (Puerto Rico referring to the results of this study); and *M. undulatus* from the Gulf of Mexico and Virginia (western north Atlantic). This genus hosts six of the total of species of macrovalvitrematids reported and six of the 10 genera recognized previous to this study. Two species of *Umbrina* have been reported as a host for macrovalvitrematid, *U. coroides* from Argentina and *U. roncadore* from the Pacific coast of Mexico.

*Ophioscion scierus* from the Pacific coast of Mexico is the third species of the family Sciaenidae reported as host of a macrovalvitrematid.

The genus *Orthopristis* has been the predominant haemulid host for the macrovalvitrematid genus *Pseudotagia*, which shows a close relationship with only haemulid and serranid fishes, and is the only macrovalvitrematid genus that does not parasitize members of the family Sciaenidae. Two species of *Orthopristis* have been reported as host of the macrovalvitrematid genus *Pseudotagia*, *O. chrysopterum* from the Gulf of Mexico and *O. ruber* from Brazil in the south Atlantic and the Gulf of Cariaco in Venezuela (Caribbean). Kohn et al. (1984) also reported a pseudotagid macrovalvitrematid from the gills of the haemulid *Haemulon sciurus*.

The serranids *Paralabrax clathratus* from California and *Diplectrum* sp. from Brazil have also been reported as hosts of pseudotagid macrovalvitrematids. *Lutjanus argentiventris* from Baja California has been the only lutjanid reported as host for a macrovalvitrematid monogenean.

Because most of the genera are monotypic and there are only a few macrovalvitrematid species described leads us to consider that there still may be many other species yet to discover. Also, except for the genus *Micropogonias*, most of the Sciaenid genera as well as the Sparidae genera are not well studied allowing us to consider that it is possible that the host range of the macrovalvitrematids is larger and perhaps less host specific.

### **Phylogeny**

The family Macrovalvitrematidae has not been the focus of any previous phylogenetic work, accounting for their placement in the family Diclidophoridae by Boeger and Kritsky (1993) based on the Mamaev (1976) hypothesis that the Macrovalvitrematidae confers a paraphyletic status to the Diclidophoridae. Their position and their phylogenetic relationship have not been proposed before this study. In addition to the genital corona of spines of the male copulatory organ these two families also share other similarities such as the number of clamps, the symmetry of the opisthaptor and the asymmetry of the clamps.

### **Coevolutionary trends**

Most of the fish families identified as hosts for members of the family Macrovalvitrematidae (Sciaenidae, Lutjanidae, Sparidae, Haemulidae and Serranidae)

have been related by the phylogenetic data of other monogeneans (Boeger and Kritsky, 2003). As mentioned above the families Sciaenidae and Haemulidae are more frequently found as hosts of the macrovalvitrematid monogeneans. But there is a marked division between the parasites and their hosts with the eight of the 10 genera and 12 species (*Macrovalvitrema sinaloense*, *Pterinotrematoides mexicanum*, *Macrovalvitrematoides micropogoni*, *Hargisia bairdiella*, *Neomacrovalvitrema argentinensis*, *Neopterinotrematoides avaginata*, *Nicolasia canosorum* and *Pseudohargisia cortesi*) parasitizing the gills of sciaenids fishes, and with only the species of the genus *Pseudotagia* parasitizing the gills of haemulid fishes. In addition, *Papillopseudotagia hubbsi* is the only macrovalvitrematid reported from a non-perciform (Order Pleuronectiformes) host fish, *Citharichthys sordidus* and possesses unique characters. The morphology of the macrovalvitrematids found on hosts other than the Sciaenidae, have distinctive characters that probably were acquired (required) to adapt to their host. For example the only macrovalvitrematid found in the sparid *Archosargus* has lost the pharynx and increased the width of their anterior end, while the ones found in the paralichthid *C. sordidus* has a significant reduction of the pharynx and the presence of papillae in the ventral valve of their clamps among other autapomorphies. The clamps of the genus *Pseudotagia* (which are the only macrovalvitrematid parasitizing the Haemulidae) are significantly different from the arrangement observed in the clamps of the macrovalvitrematids parasitizing the other host families (including Sciaenidae, Sparidae and even *Papillopseudotagia*).

The main objectives of this study are to know the fauna of macroalvitrematids of Puerto Rico, to develop a hypothesis of the phylogenetic relationships among the macroalvitrematid species and to infer some possible coevolutionary events between host-parasite relationships. This study represents the first survey of the Family Macroalvitrematidae Yamaguti (1963) from Puerto Rico (mostly from the Caribbean coast, but with some records from the north Atlantic) , the addition of new host and locality records for the family, the first revised classification based on cladistic analysis and the first attempts to reconstruct the host phylogeny, and to identify some possible coevolutionary explanations among the macroalvitrematids and their hosts.

## Chapter II.

### Collection and Taxonomic Description of members of the Family Macrovalvitrematidae Yamaguti, 1963 of Puerto Rico

**Abstract:** The Class Monogenea van Beneden, 1858 comprises a diverse group of platyhelminthes parasitic primarily on the external surfaces and gills of marine and freshwater fishes (Boeger and Kritsky, 2001). The Macrovalvitrematidae Yamaguti, 1963 is a small family of heteronchoinean monogenea, which, until this survey, was composed of 10 genera and 12 species with very distinctive morphological features. This study represents the first survey of macrovalvitrematid monogeneans from the Caribbean and this chapter will record the monogeneans of family Macrovalvitrematidae in Puerto Rico. Fishes were collected from the west and northeast coasts of Puerto Rico using a beach seine method. A total of 143 fishes of 12 genera and 18 species belonging to the families Sciaenidae, Haemulidae and Sparidae were collected and examined for monogenean parasites. With the exception of *Hargisia bairdiella* and *Pseudotagia cupida* all the species included in the family Macrovalvitrematidae Yamaguti 1963 were borrowed and compared with the material collected in this study. Behaviors of live specimens were observed including body movements, regurgitation of cecal material and oviposition. The sciaenid *Micropogonias furnieri*, the haemulid *Pomadasys corvinaeformis* and the sparid *Archosargus rhomboidalis* resulted parasitized by macrovalvitrematid monogeneans. Of the 353 monogeneans collected, 189 specimens correspond to macrovalvitrematid monogeneans of four genera and four species. This includes *Macrovalvitrema argentinensis* (Suriano, 1975) n. comb., *Neopteriotrematoides avaginata* Suriano, 1975, one new genus and species and one new species of *Pseudotagia*.



## **Introduction**

The Class Monogenea van Beneden, 1858 comprises a diverse group of platyhelminthes parasitic primarily on the external surfaces and gills of marine and freshwater fishes (Boeger and Kritsky, 2001). They are bilaterally symmetrical ranging in size from 0.03 to 20 mm long (Roberts and Janovy, 2009). The Monogenea are an ideal group for investigation of diversification and diversity for at least three reasons: they are diverse both in terms of morphology and numbers, they are generally host specific, and their phylogeny is well resolved, at least to the family level (Poulin, 2002). At present, the prevailing opinions divide the class Monogenea into two subclasses: Polyonchoinea and Heteronchoinea previously known as Monopisthocothylea and Polyopisthocothylea respectively (Boeger and Kritsky, 2001). The group of interest in this study is the family Macrovalvitrematidae Yamaguti, 1963, which have been poorly understood and which their taxonomic position is still not resolved.

## **Taxonomic Position of Macrovalvitrematidae Yamaguti 1963**

Macrovalvitrematidae is in the Subclass Heteronchoinea; Infraclass Oligonchoinea (Bychowsky, 1937); Order Mazocraeidea Bychowsky, 1937; Suborder Microcotylea Lebedev, 1972 and located inside the Family Diclidophoridae Cerfontaine, 1895. Boeger and Kritsky (1993) incorporated the Macrovalvitrematidae (Heteronchoinea) into the family Diclidophoridae since recognition would confer paraphyletic status for the Diclidophoridae. They tentatively considered the Macrovalvitrematidae synonyms of

Diclidophoridae based on Mamaev's 1976 phylogenetic hypothesis of the Diclidophoridae.

### **Heteronchoinea**

The members of the Subclass Heteronchoinea have an oral sucker, a genitointestinal canal and an opisthaptor armed with clamps or suckers and larval hooks persisting from the oncomiracidium (larval stage) Hernández-Vale (2003). The most distinctive and important organs are their attachment organs, the buccal or feeding organs and the genitalia.

### **Heteronchoinea: Macrovalvitrematidae Yamaguti, 1963**

The Macrovalvitrematidae Yamaguti, 1963 is a small family, which, until this survey, was composed of 10 genera and 12 species with very distinctive morphological features. According to the diagnosis of Yamaguti (1963) the family is mainly characterized by an anterior end with a goblet shape, armed genital sucker or bulb, or genital corona with several spines and an opisthaptor with eight (four pairs) of clamps, which are longer than wide and shaped like a miter, guitar or fire tongs. The type genus of the family is *Macrovalvitrema* Caballero y C. and Bravo-Hollis, 1955 and the members of the family are accommodated into two subfamilies by Bravo-Hollis (1982), Macrovalvitrematinae and Pterintotrematoidinae according to the clamp structure and copulatory complex. The valid species included in the family are *Macrovalvitrematoides micropogoni* (Pearse, 1949); *Macrovalvitrema sinaloense* Caballero y C. and Bravo-Hollis, 1955; *Pterintotrematoides mexicanum* Caballero y C. and Bravo-Hollis, 1955; *Hargisia*

*bairdiella* (Hargis, 1956); *Pseudotagia cupida* (Hargis, 1956); *P. clathratus* Crane, 1972; *Neomacrovalvitrema argentinensis* Suriano, 1975; *Neopteriotrematoides avaginata* Suriano, 1975; *Nicolasia canosorum* Suriano, 1975; *Pseudohargisia cortesi* Payne, 1987; and *Papillopseudotagia hubbsi* Payne, 1987.

They have been reported from the Pacific Ocean, the Gulf of Mexico and North Atlantic and the South Atlantic, from the host families Sciaenidae, Haemulidae, Serranidae, Lutjanidae and Paralichthidae. Table I-1 lists the species of macrovalvitrematids and their respective localities and host families. This study represents the first survey of macrovalvitrematid monogeneans from the Caribbean and this chapter will record the monogeneans of family Macrovalvitrematidae in Puerto Rico.

## **Morphological characterization of the Heteronchoinea and Macrovalvitrematidae**

### **I. Anterior End: Oral Cavity and Oral Suckers**

#### **Heteronchoinea**

The heteronchoineans damage their hosts by feeding on blood, a behavior recognized by Llewelyn in 1954. They often occur attached to the highly vascularized gill tissues of their hosts. The shape and accessories found in the anterior end vary according to family. They have a terminal or subterminal mouth that opens into an oral cavity that is either suctorial, as in polystomatid monogeneans of amphibians or the hexabothrid gill monogeneans of elasmobranchs, or contains two oral suckers, as in the teleost gill monogeneans (Kearn, 1998). Since they feed on blood, there is little need to move from

place to place, thus most heteronchoineans are sedentary (Kearn, 1998). According to Bychowsky (1957) the most highly evolved species are not capable of moving.

### **Heteronchoinean: Macrovalvitrematidae Yamaguti, 1963**

The goblet-shaped anterior end of macrovalvitrematids is obvious and distinctive and is one of the main characters of the family diagnosis. Yamaguti (1963) described it as a goblet shaped anterior end with a pair of large oral suckers opening into the oral cavity. Their mouth or oral disc can be terminal or subterminal when the dorsal border is slightly extended anteriorly of the ventral border. The oral disc in most or in all the macrovalvitrematid species has the same or almost the same width as the anterior end. This allows them to open this organ completely to ensure the attachment of the oral suckers inside to the gill tissue. Caballero y C. and Bravo-Hollis (1955) refer to this part of the body as “wide goblet shaped” for the two species that they described. Most macrovalvitrematid species have this wide structure that is important for attachment and feeding.

## **II. Genitalia**

### **Heteronchoinea**

The monogeneans are hermaphroditic with cross-fertilization usually taking place (Roberts and Janovy, 2009). The most distinctive structures or organs of the female genitalia of heteronchoinean monogenea are the vagina, ovary and uterus. Among these structures the ovary is easiest to see, the uterus in many examinations can be difficult to

observe, except when an egg is enclosed. Presence of a vagina is a very controversial character because, in many cases, the opening is not clearly visible. It is the only organ that can have apertures in different positions in the body. It can open laterally, dorsally, ventrally and at different levels of the body. The number of apertures can vary and they can be unarmed or armed depending on the species.

Among the male genitalia, the most important organs used as taxonomic characters are the testes and the male copulatory organ. The testes generally are spherical or ovoid and occupy the posterior portion of the body proper. In many species it is difficult to determine their shape and their number since they are obscured by the vitellaria. The copulatory organ, a more conspicuous structure, has more taxonomic value since in most cases it may be armed with several spines. The male copulatory organ of heteronchoineans is very diversified. Basically, there are two types: one with a muscular penis protruding through the genital pore and the other with a completely sclerotized formation (Bychowsky, 1957). It can be unarmed or armed with straight spines, curved spines, hook-like spines or a combination (Hernández-Vale, 2003).

In addition to those structures the heteronchoinean monogeneans usually have a single common genital atrium in which the male copulatory organ and the uterus open (Bychowsky, 1957). This atrium is located either on the ventral side of the body, posterior to the esophagus or posterior to the intestinal bifurcation. In more evolved species, an armature can be present on the upper interior surface of the atrium

(Bychowsky, 1957). The genital atrium and the male copulatory organ can be important characters in the diagnosis of a family of heteronchoinean (e.g., Macrovalvitrematidae). One common problem with these two structures, in taxonomic descriptions, is that both can be misinterpreted as the other.

### **Heteronchoinea: Macrovalvitrematidae**

The genitalia of the family Macrovalvitrematidae is distinguished by the presence of a genital corona of spines. This is similar to the genital coronet of the family Diclidophoridae. The number of spines is variable even in different specimens of the same species; and the shape and size differs slightly among the different genera of the family. This genital corona corresponds to the armature of the male copulatory organ. This has been misinterpreted in some publications as the genital atrium, others referred to this simply as genital corona. For example: Caballero y C. and Bravo-Hollis (1955) referred to this structure in *Macrovalvitrema sinaloense* as a spherical muscular copulatory organ bordered with a genital disc armed with several spines; Hargis (1956) referred to this as an armed genital atrium for *Macrovalvitrematoides micropogoni*; Suriano (1975) described it for *Neomacrovalvitrema argentinensis* as spherical with a wide muscular walled genital atrium in which was inserted six spines; and Payne (1987) referred to it in *Pseudohargisia* and *Papillopseudotagia* simply as a genital corona. The vagina is also a controversial character in the macrovalvitrematids. Some species have only one dorsal opening, others possess two lateral openings, another one lateral opening and in others the vaginal pores have not been observed. Originally the

Yamaguti's (1963) taxonomic key for the genera of the family was based on differences of the vagina. Yamaguti (1963) established that parasites with a single vagina its opening is dorsal, while the ones with two vaginae have lateral openings. According to this key the species of the Pacific possess a single vagina and the ones from the North Atlantic have two vaginae with lateral openings. But finding the vaginal pores can be difficult leading to misinterpretations in some of the macroalvitrematid species. As example Caballero y C. and Bravo-Hollis (1955) described that the species *P. mexicanum* has a large and dorsal vaginal pore, and Bravo-Hollis (1982) redescribe it as two vaginae with lateral openings posterior to the level of the copulatory organ. Suriano (1975) erected a new genus using the "absence of the vagina" as the only distinctive character. The family includes two species with one dorsal vaginal opening, six with lateral openings and four in which the vagina was not observed.

### **III. Opisthaptor**

#### **Heteronchoinea**

The opisthaptor is the principal attachment apparatus used to fix the parasite to its host. It is found on the posterior end of the body and contains a group of structures, each of which functions independently (Bychowsky, 1957). The opisthaptor exhibits great variation within the Monogenea, and many forms have been interpreted as adaptation to particular host and infection sites (Roberts and Janovy, 2009). Since the opisthaptor varies considerably among species, it is useful in distinguishing species and identifying evolutionary trends. The heteronchoinean opisthaptor is distinguished from those of other monogeneans by having suckers or attaching clamps that have different shapes from any

other family and in some cases are distinctive for a particular genus (Hernández-Vale, 2003).

### **Heteronchoinea: Macrovalvitrematidae Yamaguti, 1963**

The opisthaptor of members of the family Macrovalvitrematidae is distinct from the other heteronchoinean monogeneans. Yamaguti (1963) described the opisthaptor of the members of this family as one composed of eight (four pairs) of clamps, which are longer than wide and shaped like a miter, guitar or fire tongs. These clamps are characterized as being pedunculated as is their closest sister family (Diclidophoridae), which also has eight clamps. But in Macrovalvitrematidae the clamps are arranged symmetrically in pairs along the opisthaptor and are oriented ventrally except the last pair which is oriented posteriorly. The opisthaptor of macrovalvitrematids can have one or two kinds of clamps. It also has a hook-bearing terminal lappet.

The classification or generic key of the macrovalvitrematid was originally made (by Yamaguti, 1963) based on the vagina, but after more species were discovered this system became unworkable. The morphology of the opisthaptor then became the primary characters used in their classification. Suriano (1975) described and placed three new genera and species in the family Macrovalvitrematidae and established a relation among those species and the previous species described, but based on the similarities of the opisthaptor. She also compared the species based on the hook arrangement of the oncomiracidium. The morphology of the opisthaptor is one of the most distinctive characters of this family. This was also noticed by Bravo-Hollis (1982) when she erected



two subfamilies (Macrovalvitrematinae and Pterinotrematoidinae) using the morphology and arrangement of the opisthaptor together with the arrangement of the male genital complex. In this classification, she differentiates those macrovalvitrematids having an opisthaptor with four pairs of similar clamps (Macrovalvitrematinae) from those having an opisthaptor with three pairs of racquet-shaped clamps and one pair of small and different type clamps (Pterinotrematoidinae).

Table II-1 Genera and Species of the Family Macrovalvitrematidae Yamaguti, 1963, their synonyms, known localities, and host families

Species	Synonyms	Localities	Host Families
<i>Macrovalvitrematoides micropogoni</i> (Pearse, 1949) Yamaguti 1963	<i>Tagia micropogoni</i> Pearse, 1949; redescribed and maintained in the same genus by Hargis, 1956	(North Carolina) and Gulf of Mexico (Florida)	Sciaenidae
<i>Macrovalvitrema sinaloense</i> Caballero and Bravo-Hollis, 1955	N/A	Pacific Ocean (Mexico) South Atlantic (Brazil)	Sciaenidae and Lutjanidae
<i>Pterinotrematoides mexicanum</i> Caballero and Bravo-Hollis, 1955	N/A	Pacific Ocean (Mexico and Perú) South Atlantic (Argentina)	Sciaenidae and Lutjanidae
<i>Hargisia bairdiella</i> (Hargis, 1956) Yamaguti 1963	<i>Tagia bairdiella</i> Hargis, 1956	Gulf of Mexico (Florida)	Sciaenidae
<i>Pseudotagia cupida</i> (Hargis, 1956) Yamaguti 1963	<i>Tagia cupida</i> Hargis, 1956	Gulf of Mexico (Florida)	Haemulidae
<i>P. clathratus</i> Crane, 1972	N/A	Pacific Ocean (California)	Serranidae
<i>Neomacrovalvitrema argentinensis</i> Suriano, 1975	N/A	South Atlantic (Argentina)	Sciaenidae
<i>Neopterinotrematoides avaginata</i> Suriano, 1975	N/A	South Atlantic (Argentina)	Sciaenidae
<i>Nicolasia canosorum</i> Suriano, 1975	N/A	South Atlantic (Argentina)	Sciaenidae
<i>Pseudotagia rubri</i> Luque et al. 1993	N/A	South Atlantic (Brazil)	Haemulidae and Serranidae
<i>Papillopseudotagia hubbsi</i> Payne, 1987	N/A	Pacific Ocean (California)	Paralichthyidae
<i>Pseudohargisia cortesi</i> Payne, 1987	N/A	Pacific Ocean (California)	Sciaenidae

## Review of Literature

Pearse (1949) described the first member of this family, which he named *Tagia micropogoni*, from the gills of *Micropogonias undulatus* (Linnaeus 1766). It was later redescribed by Hargis (1956) but not reassigned. In the same paper Hargis (1956) described two additional species in the genus *Tagia*, *T. bairdiella* and *T. cupida* from the gills of *Bairdiella chrysoura* (Lacepède, 1802) and *Orthopristis chrysopterus* (Linnaeus, 1766) respectively. All three species were collected from fishes found in the north Atlantic. The next two species were described by Caballero y C. and Bravo-Hollis (1955) who placed them in two new genera, *Macrovalvitrema sinaloense* and *Pterinotrematoides mexicanum*, both from *Micropogonias ectenes* (Jordan and Gilbert, 1882) from the Pacific coast of Mexico. They placed their two new species in the superfamily Diclidophoroidea Price, 1936, family Microcotylidae Taschenberg, 1879 and erected the subfamily Pterinotrematinae to accommodate these two new species along with *Pterinotrema macrostomum* Caballero et al., 1954 which was designated as the type genus.

Bychowsky and Nagibina (1959) created the suborder Pterinotrematinea and the family Pterinotrematidae, for the single genus *Pterinotrema* removing *Pterinotrema macrostomum* from association with *Macrovalvitrema* and *Pterinotrematoides*. Yamaguti (1963) in his monograph on the Monogenea, erected the family Macrovalvitrematidae in the suborder Polyopisthocotylea and superfamily Diclidophoroidea to include those species which possess the following characters: “Body cylindrical or subcylindrical; anchor-bearing terminal lappet; eight clamps longer than wide, shaped like a miter, fire-tongs or guitar, variously modified in different genera; goblet shaped anterior end with a pair of large oral suckers opening into oral cavity; intestinal crura with diverticula; testes postovarian, single or divided into a number of comparatively large follicles; armed genital sucker or bulb, or genital coronet present; pore bifurcal or postbifurcal; ovary

curved and pretesticular; genito-intestinal canal present; vagina present; and vitellaria almost entirely co-extensive with intestine.”

In this family he included the species from the Pacific coast of Mexico (*M. sinaloense* and *P. mexicanum*) described by Caballero y C. and Bravo-Hollis (1955) and the ones from the north Atlantic described by Pearse (1949) *Tagia micropogoni*, and Hargis (1956) *Tagia bairdiella* and *T. cupida* but removed these three from the genus *Tagia* erecting the genera *Macrovalvitrematoides*, *Hargisia* and *Pseudotagia* for each species respectively. He designated *Macrovalvitrema sinaloense* as the type genus of the family and established a key for the five genera based on the number and location of vaginae.

Those species with a single vagina opening mid-dorsally include:

*Macrovalvitrema* having all clamps guitar-shaped with equatorial sclerites, without spines or papillae on external and internal surfaces of each valve.

*Pterinotrematoides* with only the dorsal clamps guitar shaped, lacking equatorial sclerites.

The species with double vaginae, with paired lateral openings belong to the genera:

*Hargisia* with a single testis, region of vaginal openings with plicated placodes, clamps not uniform in structure with posterior-most pair rounded unlike other three pairs which are shaped like fire-tongs.

*Pseudotagia* with multiple testes, region of vaginal openings without plicated placodes and clamps similar in structure and clamps not guitar- or fire-tongs-shaped, but serrate on lateral margins.

*Macrovalvitrematoides* with multiple testes, region of vaginal openings without plicated placodes and clamps similar in structure and guitar-shaped, not serrate on lateral margins.

Suriano (1966) reported *P. mexicanum* as part of a study of the parasitic fauna of *Micropogonias opercularis* Quay and Gaimard, 1825 (synonym of *M. furnieri*) to discuss some zoogeographic aspects of the south Atlantic. Crane (1972) described the species *Pseudotagia clathratus* collected from the serranid *Paralabrax clathratus* (Girard, 1854) from California and placed it in the Macrovalvitrematidae. Suriano (1975) adopted the classification of Yamaguti (1963) for the family Macrovalvitrematidae; and added three new genera and species collected from Mar del Plata, Argentina (south Atlantic), *Neomacrovalvitrema argentinensis*, *Neopteriotrematoides avaginata* collected from *Micropogonias furnieri* (Desmarest, 1823) and *Nicolasia canosorum* from *Umbrina canosai* Berg, 1895. Suriano (1975) placed these new genera in the Macrovalvitrematidae based on the anatomy of the opisthaptor. She allied *Neomacrovalvitrema* with the genera *Macrovalvitrema* and *Macrovalvitrematoides*; and *Neopteriotrematoides* and *Nicolasia* with the genera *Pteriotrematoides* and *Hargisia*. In addition, Suriano (1975) remarked that the anatomy and morphology of the opisthaptor (particularly the clamps) of the genus *Pseudotagia* do not resemble the other genera of the family and argued that this is the only genus that does not parasitize the gills of a sciaenid.

Lebedev (1979) accepted the creation of the family Macrovalvitrematidae by Yamaguti, 1963, but transferred it to the suborder Discocotylinea Bychowsky, 1957 together with the families Diclidophoridae, Anchorophoridae and Anthocotylidae (Boeger and Kritsky, 1993). Mamaev and Lebedev (1979) preserved the family in the Order Mazocraeidea Bychowsky (1957) and suborder Discocotylinea (as designated previously). Bravo-Hollis (1982) agreed with the order and suborder designation of Lebedev (1979) and Mamaev and Lebedev (1979), but used the suffix “-iformes” suggested by Caballero and Bravo (1961) for the order instead of “-idea”

(Mazocraeiformes). She also erected two subfamilies (Macrovalvitrematinae and Pterinotrematoidinae) based on the morphology and arrangement of the opisthaptor and male genital complex with the Macrovalvitrematinae having an opisthaptor with four pairs of structurally similar clamps and genital atrium armed with several spines arranged radially. The genera included are *Macrovalvitrema*, *Macrovalvitrematoides*, *Neomacrovalvitrema* and *Pseudotagia*. With the subfamily Pterinotrematoidinae she grouped those genera having an opisthaptor with three pairs of raquet-shaped clamps and one pair of small macrovalvitrematiform type clamps; and a cirrus bulb and cirrus armed with three pairs of different hooks arranged bilaterally. In this subfamily she includes the genera *Pterinotrematoides*, *Hargisia*, *Neopterinotrematoides* and *Nicolasia*.

Kohn et al. (1984) reported, studied and briefly described some monogenean parasites of *Haemulon sciurus* (Shaw, 1803), including the macrovalvitrematid *Pseudotagia cupida* (Hargis, 1956). Tantalean et al. (1985) reported Perú as new locality record for *Pterinotrematoides mexicanum*. Payne (1987) described two new genera and species of the family Macrovalvitrematidae from California (north Pacific), *Papillopseudotagia hubbsi* from *Citharichthys sordidus* (Girard, 1854) and *Pseudohargisia cortesi* from *Micropogonias megalops* (Gilbert, 1890), and placed them in the subfamilies Macrovalvitrematinae and Pterinotrematoidinae respectively. Kohn et al. (1989) found, identified and reported two macrovalvitrematids as *Macrovalvitrema sinaloense* and *Pterinotrematoides mexicanum* from the gills of *Micropogonias furnieri*. They argued that the types *Neomacrovalvitrema argentinensis* and *Neopterinotrematoides avaginata* described from the same host fish by Suriano (1975) must be reviewed considering that the only differential character “absence of vagina” is not a

sufficient character to separate genera. They agreed with Gussev's statement (1976) that "the words 'lacking' or 'presence' of vagina should be avoided in differential diagnosis.

Spencer and Gibson (1990) made a list of old and recently erected monogenean genera-group names not included in Yamaguti's 1963, *Systema Helminthum*. In this list the macrovalvitremitid monogeneans included *Neomacrovalvitrema argentinensis*, *Neopteriotrematoides avaginata* and *Nicolasia canosorum* described by Suriano (1975) and *Pseudohargisia cortesi* and *Papillopseudotagia hubbsi* described by Payne (1987). Kohn et al. (1992) reported new host records and localities of some Monogenea from Brazilian marine fishes, including *Pseudotagia cupida* from a serranid species of the genus *Diplectrum* and *Macrovalvitrema sinaloense* from *Pomatomus saltatrix* (Linnaeus, 1766) (Pomatomidae). Luque *et al.* (1993) described *Pseudotagia rubri* on *Orthopristis ruber* (Cuvier) from the Brazilian coast (south Atlantic).

The phylogenetic analysis of Boeger and Kritsky (1993) does not support the inclusion of the families Macrovalvitremitidae, Diclidophoridae, Anchorophoridae and Anthocotylinea in the suborder Discocotylinea and they proposed to transfer the Diclidophoridae to the suborder Microcotylinea. They also incorporated the Macrovalvitremitidae and Anchorophoridae tentatively into the Diclidophoridae considering them as synonyms of Diclidophoridae since this would confer paraphyletic status to the Diclidophoridae. This is based on the evolutionary hypothesis of Mamaev's (1976) that both the Macrovalvitremitidae and Anchorophoridae have their origins within the Diclidophoridae and share common ancestors with separate diclidophorid genera.

Kohn and Cohen (1998) made a list of species, host and geographical distributions of south American Monogenea. In this list they report some macroalvitrematid species: *Neomacroalvitrema argentinensis*, *Neopteriotrematoides avaginata* and *Pteriotrematoides mexicanum* on *Micropogonias furnieri* and *Nicolasia canosorum* on *Umbrina canosoi* from Mar del Plata, Argentina; *Macroalvitrema sinaloense* and *P. mexicanum* on *M. furnieri*, *Pseudotagia cupida* on *H. sciurus*, and *P. rubri* on *Orthopristis ruber*, all from Rio de Janeiro, Brazil; and *Pteriotrematoides mexicanum* of *Micropogonias* sp. from Lima, Perú. Kohn and Pinto-Paiva (2000) make a list of freshwater and marine fishes in South America parasitized by monogeneans. In this list they reported *Pomatomus saltator* as host of *M. sinaloense*; *Haemulon sciurus* as host of *Pseudotagia cupida*, and *Orthopristis ruber* as host of *P. rubri*; *Umbrina canosoi* as host for *Nicolasia canosorum* and *Micropogonias furnieri* as the host for *Neomacroalvitrema argentinensis*, *Neopteriotrematoides avaginata* and *Pteriotrematoides mexicanum*. Santos and Carbonel (2000) discuss the role of physical factors in the distribution of the monogenean fauna in the midwestern and southwestern Atlantic. In this work they present the distributions of different genera of monogeneans in the southwestern Atlantic, Caribbean, and Gulf of Mexico. Among the genera of the family Macroalvitrematidae they located *Macroalvitrema*, *Neomacroalvitrema*, *Neopteriotrematoides*, *Nicolasia*, *Pseudotagia* and *Pteriotrematoides* in the southwestern Atlantic; *Macroalvitrematoides*, *Hargisia* and *Pseudotagia* in the Gulf of Mexico; and no genera in the Caribbean Sea.

Alves and Luque (2000) studied the metazoan parasites of *Micropogonias furnieri* from the coastal zone from the state of Rio de Janeiro, Brazil, and reported the macroalvitrematid monogeneans *Macroalvitrema sinaloense* and *Pteriotrematoides mexicanum*. Alves and Luque (2001) studied the community ecology of the whitemouth croaker *Micropogonias furnieri* and



found that prevalence and abundance of *P. mexicanum* were positively correlated with host total length. Centeno et al. (2002) compared the metazoan communities among the haemulids *Orthopristis ruber* and *Haemulon steindachneri* (Jordan and Gilbert, 1882) from the Gulf of Cariaco, Venezuela. They found a single macrovalvitremitid monogenean of the genus *Pseudotagia* on the gills of *O. ruber*, the capsalid monogenean *Encontyllabe pagrasomi* MacCallum, 1917 and two didelphophorids of the genus *Choricotyle* van Beneden y Hesse, 1863. Hernández-Vale (2003) proposed as a new genus and species a macrovalvitremitid monogeneans found on the gills of *Archosargus rhomboidalis*. Fuentes Zambrano et al. (2009) reported *Pterinotrematoides mexicanum* on the sparid fish *A. rhomboidalis* from Margarita Island, Venezuela. Fernandez et al. (2009) reported *Macrovalvitrema sinaloense* and *Pterinotrematoides mexicanum* from the gills of *Micropogonias furnieri*.

## Material and Methods

### Fresh Material

Fishes were collected from the west (beaches of Guanajibo and Boquilla in Mayagüez, and Combate, Cabo Rojo) and northeast coasts (Loiza) of Puerto Rico using a beach seine as part of a fishery study conducted by Dr. E. Ojeda Serrano of the Sea Grant Program to determine the effect of this fishing method on the fish populations near the beaches of Puerto Rico. A total of 18 fish species belonging to the families Sciaenidae, Haemulidae, Sparidae were collected for this study. Fishes were removed from the net, identified, weighed and measured for total length, placed in plastic bags by species, kept in a cooler with ice until brought to the laboratory, where they were placed in the freezer until examination. Frozen fishes were thawed just prior to their examination. Some of the fishes were allowed to be retained by the fishermen that collaborated in the study. The gills of these fishes were removed in the field and placed in small bags identified with their common or scientific name, kept in a cooler with ice until brought to the laboratory where they were frozen for later examination.

In the laboratory, fishes were thawed, the gills removed and examined for ectoparasites specifically for macroalvitrematid monogeneans using a dissection microscope. Most of the monogeneans found in the gills were fixed in formalin (10%), and a few of each species were fixed in 95% alcohol for future molecular analysis. The majority of the macroalvitrematids collected were mounted in glycerin gelatin and some of each species were stained with Gomori trichrome and/or with carmine and mounted in Canada balsam. Some of the specimens were maintained in formalin (10%) and studied without mounting. Representative specimens will eventually be deposited in the USNPC.

## Museum Material

With the exception of *Hargisia bairdiella* (Hargis 1956) Yamaguti 1963 and *Pseudotagia cupida* (Hargis 1956) Yamaguti 1963, all the species included in the family Macrovalvitrematidae Yamaguti 1963 were borrowed and compared with the material collected in this study. *H. bairdiella* and *P. cupida* were not possible to be acquired because the USNPC lost them in a previous loan. The original descriptions of these two species were used to compare with the new material. The museum material examined in this study includes:

- *Macrovalvitrema sinaloense* Caballero y C. and Bravo-Hollis 1955 -(Paratype) - Colección Nacional de Helminthos (CNHE), Instituto de Biología de la Universidad Nacional Autónoma de México” paratype CNHE No. 213-215, 1 voucher CNHE No. 231-6 and 4 vouchers CNHE No. 231-10.
- *Pterinotrematoides mexicanum* Caballero y C. and Bravo-Hollis 1955 -(Paratype) - Colección Nacional de Helminthos (CNHE), Instituto de Biología de la Universidad Nacional Autónoma de México” paratypes CNHE No. 213-216.
- *Neomacrovalvitrema argentinensis* Suriano 1975, “Museo de La Plata (MLP), División Zoología Invertebrados, La Plata, Buenos Aires, Argentina” holotype MLP No. P2.
- *Neopterinotrematoides avaginata* Suriano 1975, Museo de La Plata (MLP), División Zoología Invertebrados, La Plata, Buenos Aires, Argentina” holotype MLP No. P3.
- *Nicolasia canosorum* Suriano 1975, “Museo de La Plata (MLP), División Zoología Invertebrados, La Plata, Buenos Aires, Argentina” holotype MLP No. P4.
- *Macrovalvitrematoides micropogoni* (Pearse 1949) Hargis 1956 - (Paratype)- U.S. National Parasite Collection (USNPC), Beltsville, Maryland. holotype and two paratypes USNPC 36961

- *Pseudotagia clathratus* Crane 1972, U.S. National Parasite Collection (USNPC), Beltsville, Maryland two paratypes USNPC No. 71930-71931
- *Pseudotagia rubri* Luque, Amato and Takemoto 1993, U.S. National Parasite Collection (USNPC), Beltsville, Maryland two paratypes USNPC No. 83186-87.
- *Pseudohargisia cortesi* Payne 1987, U.S. National Parasite Collection (USNPC), Beltsville, Maryland one holotype USNPC No. 79499 and one paratype HWML No. 23641
- *Pseudohargisia cortesi* Payne 1987, Harold W. Manter Laboratory (HWML), University of Nebraska State Museum holotype USNPC No. 79499 and one paratype HWML No. 23641.

The material examined of the species *Macrovalvitrematoides micropogoni* and *Pseudotagia clathratus* were microphotographs taken by Patricia Pilitt of the holotype material deposited in the US National Parasite Collection (USNPC). The paratype slides of these species (which is the only type of material loaned by this museum) were not available.

### **Additional Material**

Additional specimens collected in Puerto Rico and identified as *Pterinotrematoides mexicanum* by Dr. E. H. Williams and L. Bunkley-Williams from the sciaenid *Ophioscion adjustus* and the sparid *Archosargus rhomboidalis* from Guayanilla Bay, Guayanilla and La Parguera, Lajas respectively. Although part of the *A. rhomboidalis* material collected by them is also deposited in this museum, material from their personal collection was used to compare with the fresh material collected in this study. Hernandez-Vale (2003) examined these same specimens and proposed as a new genus and species in her master's thesis.

## Live specimens

Another sample of fishes (*Archosargus rhomboidalis*, *Micropogonias furnieri*, *Pomadasys corvinaeformis*, and *Bairdiella ronchus*) was captured by fishermen using the same, beach seine, technique. The gills of these fishes were removed in the field and treated as previously described except that they were examined immediately on return to the lab. The fishes were allowed to be retained by the fishermen.

In this part of the study, Monogeneans were delicately removed from the gills using a thin camel hair paintbrush to avoid damage and were transferred to a small plastic plate of 2.54 cm of diameter by 0.16 cm of height containing sterilized sea water. Behaviors were then observed including body movements, the regurgitation of cecal material (digested blood) and oviposition (as many then began to lay eggs). In addition, their most important morphological characters were observed in the live and active states. After 12-24 hours the worms were removed from the plate and fixed in buffered formalin, and eggs placed in another plate with new sterilized sea water. These plates were placed individually in plastic petri dishes with covers and kept at 19-20°C. The eggs were transferred daily to new sterilized water to avoid the growth of bacteria, fungi or protozoans. The development of the oncomiracidia (larvae) in the interior of the eggs, the hatching, the shape and the behavior of the larvae was observed and documented with photographs and video using Nikon ® coolpix L20 and Olympus ® cameras placed to the ocular lens of the microscope.

The worms and/or eggs collected were drawn with the aid of a drawing tube, studied with light microscope using Nomarski technique, photographed with Spot Insight Color and measured with their Spot Advanced Program and with an ocular micrometer. All measurements are in micrometers unless otherwise stated.

## Results and Discussion

### Host Families Selection

Fishes in families in which macroalvitrematid monogeneans had previously been reported (Sciaenidae, Haemulidae, Sparidae, Serranidae and Lutjanidae) were examined. In the beach seine project, fishes from the families Serranidae and Lutjanidae were not captured and thus not examined in this study, but previous studies of metazoan parasites of groupers and snappers of Puerto Rico by Medina-Ríos (2008) and Bosques-Rodríguez (2004) respectively did not report the presence of any macroalvitrematid. Nor had Bunkley-Williams and Williams found these parasites in 37 years of examining these fishes.

### Collection, Examination, Species Richness and Comparison with Previous Records

A total of 143 fishes of 12 genera and 18 species belonging to the families Sciaenidae (seven genera and eight species), Haemulidae (three genera and seven species) and Sparidae (two genera and three species) were collected and examined for monogenean parasites. Appendix I-1 lists the host family and species, the collection locality, collection date, examination date, species richness, parasite group or taxa, parasite species, parasite species intensity and the slide or vial # containing the material collected. Although all three families had members infected by macroalvitrematid monogenean, only one species per family were hosts. These species are the sciaenid *Micropogonias furnieri* (Desmarest, 1823), the haemulid *Pomadasys corvinaeformis* (Steindachner, 1868) and the sparid *Archosargus rhomboidalis* (Linnaeus, 1758).

From all the fishes examined a total of 392 parasites distributed among Platyhelminths (Digenean, Monogenean and Cestodes), Hirudineans (leeches), Nematodes, Copepods and

Isopods were collected. Of all these parasites 353 (90%) were monogeneans. Among the monogeneans collected were 145 (37%) polyonchoineans of at least two different genera and 208 (53%) heteronchoineans. The Heteronchoinean monogeneans collected belong to: Family Diclidophoridae (17 specimens of at least three genera), Family Microcotylidae (one specimen) and the Family Macroalvitrematidae with 189 (48%) specimens of four genera and four species. The macroalvitrematid monogeneans included two previously reported species, one new genus and species and one new species.

Sciaenidae is the host family with the majority of macroalvitrematid species reported, hosting eight of the 10 currently valid genera, with six reported from the host genus *Micropogonias*. In the present study, 14 of the 40 specimens of *M. furnieri* examined were parasitized by two species of macroalvitrematid monogeneans, while the rest had only one. In addition, four leeches (*Trachelobdella lubrica*), two nematodes, one digenean and one unknown cyst (embedded in the gill tissue), containing a large number of small unknown platyhelminths (discovered by L. Bunkley-Williams) were also found on this fish species. In addition, it is interesting to note that the *M. furnieri* parasitized by leeches had either no macroalvitrematids or had only the smaller of the two macroalvitrematid species (*P. avaginata*). Species of the sciaenid genera *Bairdiella* and *Umbrina* have also been previously reported as hosts for macroalvitrematid monogeneans (Hargis, 1956, Suriano, 1975, and Bravo-Hollis, 1982). In Puerto Rico the only species of genus *Bairdiella* found is the Ground croaker, *B. ronchus* (Cuvier, 1830). Nine specimens were examined during this study and only 54 polyonchoinean monogeneans of the genus *Rhamnocercus*, three diclidophorids, of the genus *Pedocotyle* and one digenean were collected. The two species of *Umbrina* found in Puerto Rico, the Striped drum *U. broussoneti* Cuvier, 1830 and the Sand drum *U. coroides* Cuvier, 1830 were also examined, but only 34 polyonchoinean monogeneans and one digenean were found. The Jamaica weakfish, *Cynoscion jamaicensis*

(Vaillant and Boucourt, 1883); the Shorthead drum, *Larimus breviceps* Cuvier, 1830; the Southern Kingcroaker, *Menticirrhus americanus* (Linnaeus, 1758) and the little croaker *Stellifer colonensis* Meek and Hildebrand, 1925 were other sciaenids examined, but no macroalvitrematid monogeneans were found. Only polyonchoinean monogeneans were found in *M. americanus*.

In the family Haemulidae, genera *Orthopristis* and *Haemulon* have previously been reported as hosts for the macroalvitrematids of genus *Pseudotagia* in Brazil (Kohn et al., 1984; and Luque et al. 1993), but none of the six specimens of *H. sciurus* examined in this study were infected. None of the other species of the genus *Haemulon* examined had macroalvitrematid monogeneans. They were only parasitized by polyonchoinean monogeneans. Dowgiallo (1979) studied the parasites of the French grunt *Haemulon flavolineatum* (Desmarest, 1823) from La Parguera, Puerto Rico and only found polyonchoinean and some diclidophorid monogeneans. In the present study, the gills of 18 roughneck grunts, *Pomadasys corvinaeformis*, were examined and 49 specimens of a new species of pseudotagid macroalvitrematid, 10 diclidophorids of two genera, one polyonchoinea monogenean, one unknown and damaged heteronchoinean and two leeches were found. The diclidophorids found will be included as an outgroup for the phylogenetic analysis (Chapter II).

Of the Family Sparidae, Fuentes *et al* (2009) reported *Archosargus rhomboidalis* as a host of macroalvitrematid monogeneans from Isla Margarita, Venezuela (also in Caribbean waters). But the identification of the macroalvitrematid species as *Pterinotrematoides mexicanum* was not correct. Based on their description and drawings, these monogeneans appear the same as were collected from this host in Puerto Rico, but are a new genus and species. In the present study, 25



macrovalvitremitid specimens were found from 24 *A. rhomboidalis* which also had 9 polychaete monogeneans, one heteronchoinean monogenean identified as *Microcotyle archosargi*, 10 cestode larvae, eight copepods, three leeches and two isopods of the genus *Gnathia*.

The families Serranidae and Lutjanidae were the other two families reported as hosts for macrovalvitremitid monogeneans. Crane (1972) and Kohn et al. (1984) respectively recorded the genera *Paralabrax* and *Diplectrum* as host of *Pseudotagia*. Bravo-Hollis (1982) recorded *Lutjanus argentiventris* together with other sciaenid fishes as host of two macrovalvitremitid monogeneans of the genera *Macrovalvitrema* and *Pterinotrematoides*. As mentioned above no members of these families were examined in this study; however, Medina (2008) studied the parasites of 116 groupers of 16 species and Bosques (2004) the parasites of 131 snappers of 13 species of Puerto Rico and they did not find any macrovalvitremitid

### **Taxonomic Description of Macrovalvitrematid Monogeneans collected from Puerto Rico**

In this study, four species belonging to the family Macrovalvitrematidae were collected and identified two species that were previously reported from Argentina both from the same host (*Micropogonias furnieri*), *Neomacrovalvitrema argentinensis* and *Neopterinothrematoides avaginata*. For the first new combination (*Macrovalvitrema argentinensis* will be proposed. One new genus and species from *Archosargus rhomboidalis* and one new species of the genus *Pseudotagia* from *Pomadasys corvinaeformis* will be described. In this section, the morphologically important characters of these four species will be described and differences or similarities of other macrovalvitrematid species will be compared. In addition these specimens were compared with museum material from all the other species of the family in order to identify and/or differentiate them. Also for each species, intensities, mean intensities and prevalences will be reported.

Taxonomic Summary of *Macrovalvitrema argentinensis* (Suriano 1975) new combination.

Synonyms: *Neomacrovalvitrema argentinensis* Suriano 1975

**Material examined:** 41 specimens

**Hosts Examined:** 40 *Micropogonias furnieri* (whitemouth croaker) (Sciaenidae)

**Host localities:** 35 from Guanajibo and Boquilla, Mayagüez, Puerto Rico (Caribbean) and 5 from Vacía Talega, Loíza, Puerto Rico (Atlantic)

**Hosts infected:** Total 26 (21 hosts from Mayagüez with 27 parasites; and 5 from Loíza with 14 parasites)

**Mean Intensity:** 1.28 from the Mayagüez fish and 2.8 from the Loíza fish for an overall mean intensity of 41 parasites in 26 hosts infected = 1.58;

**Prevalence:** For the Mayagüez collection 60%, for the Loíza collection 100%, for an overall prevalence of 65%

**Comparative Material:** Holotype material of *N. argentinensis*, Helminthological Collection, La Plata Museum, Argentina #P2 and Paratype material (213-215) of *Macrovalvitrema sinaloense* one slide # 314, 5 vouchers (101-102) of *M. sinaloense* “Colección Helmintológica del Instituto de Biología UNAM, Mexico.

***Macrovalvitrema argentinensis* (Suriano 1975) n. comb**

**Description:** (Based in 12 specimens; 6 measured) Body of Puerto Rican specimens is similar to *Macrovalvitrema* Caballero y C. and Bravo-Hollis, 1955 and *Neomacrovalvitrema* Suriano, 1975, 1,601  $\mu\text{m}$  long (1,140-2410; n= 6) by 156.7  $\mu\text{m}$  wide (70-230; n= 6) (Figures 1a and 1b). The **anterior end** has a wide and undulated oral disc bordered by several glands and a pair of well developed muscular oral suckers inside the oral cavity (Figure 1c and 1d). At longitudinal midline of the body, the dorsal rim of the oral disc has a small protuberance, while the ventral rim folds inward (Figure 1c). This oral disc can open completely allowing the oral suckers to contact and make suction with the substrate (Figure 1e).

**Digestive System**

The **pharynx** is oval and located posterior and between the oral suckers but in dorsal position as is the rest of the digestive tract. In live specimens it is in constant movement making the food (blood) moves through all the digestive tract. Figure 1f shows the egestion of the haematin residue (Kearn, 1998). When trying to remove one worm from the gill filaments, egestion of fresh blood (still red) from the pharynx was observed. The **intestinal bifurcation** occurs at the level of the genital aperture. The intestinal caecae extend to the posterior end of the body just anterior of the opisthaptor where they rejoin and along with the vitelline follicles enter the opisthaptor and extend dorsally in a single branch almost to the posterior most pair of clamps (Figure 2a).

## Genitalia

The **genital corona** of the male copulatory organ is located ventral and slightly right of the body midline. It is spherical, 44.5 $\mu$ m (41-48; n= 5) in diameter with a muscular base and armed with 7-11 fork-shaped (bifurcated) spines 14-16 $\mu$ m arranged radially (Figure 2b). The distal end of these spines curves inward and terminates with sharp tips. The bifurcated basal end is enlarged, flat and embedded in the muscular base. The genital opening lies anterior to the genital corona on the right side of the body. It has two lips which open when the corona extends out, during oviposition, or when vitelline material is eliminated (Figures 2c, 2d). The body has an invagination at the level of the genital opening and both the genital opening and the genital corona are oriented upward (anteriorly) at an angle of 45°. (This can only be seen when the worm is oriented on its side as in Figures 2e-2f). Ten round **testes** were observed post ovarian occupying the posterior of the body proper (Figure 2g).

The **ovary** is oval and large (2h) and is located in the medium intercecal zone measuring 112  $\mu$ m long and reaching almost the anterior part of the testicular field. The vitelline reservoir, the genitointestinal canal and the mehlis gland were not observed in the specimens studied. A vagina was not observed in any of the specimens examined.

## Opisthaptor

The **opisthaptor** is large, well developed and with eight pedunculated guitar-shaped clamps arranged symmetrically in pairs. The first (anterior) three pairs extend ventrally and the last posteriorly (Figure 3a). The first (anteriormost) pair of clamps is smaller than the rest and share a common short peduncle. A slender muscle band arises from the posterior end of the body,

posterior of the testes, and radiates into the opisthaptor, attaching to the mid-zone of each valve (Figure 3b). Between and dorsal of the last pair of clamps is a **lappet** with two pair of hooks (Figure 3c, 3d). The center pair measure 20µm (17.9- 21; n= 4) long. In many of the specimens an oval domus was observed instead of the pair of lateral hooks (Figure 3e).

Each pair of clamps appears as mirror images of each other (Figure 3f). The clamps are highly muscular and sclerotized, composed of dorsal and ventral valves each with various sclerites and a strangulation or constriction at the middle level (Figure 4a). These two valves are hinged together at the base and articulate open and closed to attach to the gill filaments (Figure 4b). They were observed attached to different locations on the gill filaments such as the tip, the edges and both sides of the hemibranchs.

The **dorsal valve** of the three large clamps is slightly larger and is composed by two kinds of sclerites, two lateral sclerites arranged symmetrically and one median sclerite (Figure 4c). The **lateral sclerites** are composed of two segments separated slightly below the level of the strangulation (Figure 4d, 4e). The basal or proximal segment terminates below the level of the strangulation with a slight curve oriented toward the inside of the valve. The distal segment of the lateral sclerites forms a semicircle that terminates distally in a short hook that articulates with the distal bifurcation of the median sclerite. At the level of the strangulation the proximal end of this sclerite segment curves into the center of the valve extending almost to the median sclerite of this valve. The **median sclerite** bifurcates at both ends. Distally it ends in two short curved tips that protrude slightly from the edge and articulate with the distal lateral sclerites (as described above). Basally, it broadens ending in larger curved tips articulating with two holes in the basal sclerite.

The interior of the dorsal valve appears to be covered mostly by glands except at the base of the median sclerite, which has both sides covered by muscular bands (Figure 4c).

The **ventral valve** although slightly smaller than the dorsal one, has a more complicated skeleton. It is asymmetrical and composed of a continuous lateral sclerite, a plate and two pairs of accessory sclerites. The **lateral sclerite** is a single piece with the anterior (distal) part wider than the proximal part. At the level of the strangulation, each side of the lateral sclerite folds inward to form one accessory sclerite which is oriented toward the center of the valve (Figure 5a). The length and the width of these two pieces are different.

The proximal part of the lateral sclerite articulates with the edges of a basal sclerite. This valve has a plate that covers almost the whole extent of the valve (Figures 5b, 5c). The anterior part of this plate is round, 38  $\mu\text{m}$  wide and narrowing at the level of the strangulation to 29  $\mu\text{m}$  and increasing again in the posterior to 49  $\mu\text{m}$  wide. At the posterior, the sides of the plate are asymmetrical. On one side it extends almost to the end of the lateral sclerite, then folds around the lateral sclerites, reaching the other side in a curved accessory sclerite (Figures 5c and 5d respectively). The other side of the plate has a fissure below the level of the strangulation (Figures 4a and 4b). The edge of this side of the plate joins the lateral sclerite prior to reaching the basal sclerite, folds around this and also reaches the other side in a small accessory sclerite (Figure 5b). Medially the plate does not reach the end of the valve, producing what appears as a space between them (Figure 4c).

The **basal sclerite** is U-shaped and is mostly oriented toward the dorsal valve. In this side of the clamp, the basal sclerite has a pair of holes in which the median sclerite of the dorsal valve

articulates (Figures 4d and 4e). The external side of this valve possesses a muscular patch delimited by three thin arc-shaped sclerites (accessory sclerites) next to each other, giving the appearance of a trilobed muscular pad (Figure 5b, 5f)).

The pair of small clamps possesses a similar configuration of the sclerites as the larger clamps. They differ only by lacking the conspicuous “trilobed muscular pad” described above.

### **Oviposition and development of eggs**

During the examination of the gills oviposition of the egg, and how it falls between the gill filaments was observed. The eggs 157.5  $\mu\text{m}$  long (150-165; n= 4) by 79  $\mu\text{m}$  (60-110; n= 4) possess one straight filament at each pole 88  $\mu\text{m}$  (70-100; n= 5) long (Figures 6). Total length of the eggs is 245.5  $\mu\text{m}$  long (220-265; n=4). On the day of the oviposition fertilized eggs are almost full of vitelline cells except for a small circular space at center. Three days after the oviposition vitelline cells were observed to have migrated to opposite poles. By the fourth day the vitelline cells were observed arranged in a line at along the egg (Figure 6a). At the fifth day the larva is well defined and was observed moving inside of some eggs (Figure 6b). The larvae began to hatch on the seventh day and the last one hatched on the 10<sup>th</sup> day (Figure 6c).

### **Description of the larvae: Morphology and Behavior**

The larva (Figures 6b-d) has cilia over most of its body except the anterior end and the sides of the attachment organ. There are no eye spots. The digestive apparatus is distinct, with a round mouth at the anterior end, leading to a small spherical pharynx opening into a sac-shaped intestine at the center of the body extending until just anterior of the attachment organ. The attachment organ has six pair of hooks, is separated from the rest of the body by a slight constriction and is

cone-shaped posteriorly. Just before hatching, the larva moves toward the pole of the egg nearest the anterior part of the body and with body contractions and ciliary beats alternates with this pushing behavior and quiet periods for a few seconds until the egg begins to split at the level of the operculum. The larva then begins to leave the egg first the body proper, followed, after several body contractions, by the posterior end (attachment organ), and after several additional body contractions, the anterior end finally emerges. As soon the larva hatches from the egg, it starts to swim rapidly, pausing for a few seconds, then swimming rapidly again. During one such pause it was possible to observe the hooks in the attachment organ arranged almost radially.

#### **Taxonomic Note:**

The macrovalvitrematid fauna collected from Mayagüez, Puerto Rico (Caribbean) consistently were composed by two species *Macrovalvitrema argentinensis* (n. comb) and *Neopteriotrematoides avaginata*. In 14 of the 35 fishes examined from the Mayagüez area, both species were found together, while the other 21 fishes infected present only one or the other with *N. avaginata* having the higher prevalence (77%) than *M. argentinensis* (60%).

This study includes the examination of five fishes from Vacía Talega, Loíza on the north coast of Puerto Rico that resulted in the collection of 14 of only the single species, *Macrovalvitrema argentinensis* (n. comb). None of the five fish were parasitized by *P. avaginata*. Although these worms were smaller (most juveniles) they share a similar genital corona and opisthaptor arrangement as *M. argentinensis*. However, one adult specimen was observed in the microscope before it was fixed and mounted. This specimen was observed from the lateral sides between the genital corona and ovary and a large pore was observed which seems to coincide with the description of vaginal pores found in *Macrovalvitrematoides micropogoni* (Pearse, 1949). *Macrovalvitrematoides micropogoni* is recorded from *Micropogonias undulatus* from the North



Atlantic. This species is similar to *Macrovalvitrema* in the opisthaptor and genital corona and, according to Hargis' (1956) description, possesses large vaginal pores on both margins between the level of the genital corona and ovary.

This vaginal pore was not observed in the specimens mentioned above after they were fixed and mounted or in any of the other specimens studied. Possibly the parasites found in *M. furnieri* from the north coast of Puerto Rico, could be a species of *Macrovalvitrematoides*. Since the examination of the material from the north coast was inconclusive, the specimens will be considered to be specimens of *Macrovalvitrema argentinensis*. The description and the measurements of *M. argentinensis* above were made with only the material collected from Mayagüez.

### Remarks

The general morphology of these worms, their measurements and clamp morphology agree with the description of *Neomacrovalvitrema argentinensis*. Despite mounting and observing both live and preserved worms in the lateral position, a vagina was never observed in any specimen consistent with the original description of *Neomacrovalvitrema argentinensis*. In addition, these specimens parasitize the same host; have the same number of testes (10-14), and same number, size and shape of coronal spines (7-11).

*Neomacrovalvitrema argentinensis* most closely resembles *Macrovalvitrema sinaloense* but differs because *M. sinaloense* (based on the modified description of Bravo-Hollis, 1982) has an average of 12 coronal spines 18-36 testes and possesses a vagina (Table I-2). In addition *M. sinaloense* has been reported from several different species of fishes. Caballero y C. and Bravo-Hollis (1955) described *M. sinaloense* originally from the sciaenid fish *Micropogonias ectenes*,

from the Pacific coast of Mexico. Later Bravo-Hollis (1982) reported this species from the sciaenids *Micropogonias megalops*, *Umbrina roncadore* and *Ophioscion adustus*, also from the Pacific coast of Mexico and California (the last one). They also identified material collected from the lutjanid *Lutjanus argentiventris* from the Pacific coast of California as *M. sinaloense*.

Suriano (1975) erected the genus *Neomacrovalvitrema* for the species *N. argentinensis* using the “absence of vagina” as the only character to differentiate this genus from the genus *Macrovalvitrema*. Although in the material from this study from Puerto Rico the vagina was never observed, this single character is not enough to establish a new genus. Kohn et al. (1989) also considered it an insufficient character and they sustained the argument based in Gusev’s statement (1976) who says “lacking or presence of a vagina should be avoided in differential diagnosis”. Based in this and considering that the two species have sufficient characters to differentiate them, a new combination, *Macrovalvitrema argentinensis* (Suriano 1975), is proposed.

The material reported as *Macrovalvitrema sinaloense* by Kohn et al. 1989 (Table I-2) was from *Micropogonias furnieri* from the south Atlantic, had 7-10 coronal spines and, although the worm lengths reported were a bit smaller, they fell well within the range of size of *Macrovalvitrema argentinensis*. Unfortunately they did not report the number of testes and stated that they observed a dorsal vaginal pore. This material as the rest of macrovalvitrematid material collected from *M. furnieri* in Brazil (Kohn, 1984; Kohn et al., 1992; Alves and Luque, 2000 and 2001) has been deposited in the museum of the “Instituto Oswaldo Cruz”. The material of Kohn et al., 1992, Kohn (1984) as well the material of Luque (2000) were requested for examination but was never

sent. Without being able to see the specimens deposited in this museum in this study it is impossible to say if they were misidentified and actually are *Macrovalvitrema argentinensis*.

The reports in the present paper of *Macrovalvitrema argentinensis* (Suriano 1975) n. comb. represent new locality records for this species. Also, the new combination establishes the genus in the south Atlantic (from Mar del Plata, Argentina and possibly Brazil) and Caribbean for the first time.

Some discrepancies between the descriptions of Suriano (1975) and Caballero y C. and Bravo-Hollis (1955) need to be addressed:

**First:** Suriano (1975) refers as genital atrium to the genital corona which she described as rounded, muscular and armed by several spines (7-11). Caballero y C. and Bravo-Hollis (1955) defined the same structure in *M. sinaloense* but as the copulatory organ, with no cirrus bulb, and described it as spherical, muscular, and surrounded by 10-12 spines. Bravo-Hollis (1982) described it as an atrial bulb armed with a crown of spines. The study of live specimens demonstrated that this structure is part of the male genital copulatory organ. The spines, which are arranged radially and oriented toward the center of the corona, turn out when the muscular base extends it out to the genital aperture causing the movement of the lips of the genital aperture toward the side (see Figures 2c-2f).

**Second:** Although the Suriano (1975) descriptions of the genital spines coincide with the orientation and shape of the worms here described, neither the description nor the drawing refers to them as bifurcated. Their genital spines are bifurcated at the basal (see figure) or fork-shaped as Bravo-Hollis (1955) described for *M. sinaloense* “en forma de horquilla” (hairgrip or hairpin). The genital spines of the new material collected from Puerto Rico, has the same shape.

**Third:** Suriano (1975) did not discuss or describe the characteristic shapes of the dorsal and ventral rim of the oral disc (Figure 1c-1e) which has a protuberance and an inward fold respectively.

**Fourth:** The plate that is found in the ventral valve of the three pair of large clamps was not described or drawn by Suriano (1975). Although *M. sinaloense* has the same plate as described here, it was not drawn or described by Caballero y C. and Bravo-Hollis (1955). Additionally, none of those descriptions note the asymmetry of the plate or the mirror image of the clamps.

**Fifth:** And finally, according to the drawings of Suriano (1975), the dorsal valve has a pair of straight lateral sclerites in its proximal part, but in observations of their holotype the curved ends below the level of the strangulation of the clamp were clearly visible as occurs in the specimen collected in this study (see Figures 4c-4d)).

Table II-2 Comparison of the two species of the genus *Macrovalvitrema* Bravo-Hollis (1982)

	Original Specimen Paratype	Specimen 2 voucher	<i>Macrovalvitrema sinaloense</i> Bravo-Hollis (1982) Specimen 3 Specimen 4 Specimen 5			<i>M. sinaloense</i> Kohn et al. (1989)	<i>Macrovalvitrema argentinensis</i> n. comb. Suriano (1975) Holotype	<i>M. argentinensis</i> (Suriano 1975) n. comb. New Material
<b>Host</b>	<i>Micropogonias ectenes</i>	<i>M. megalops</i>	<i>Ophioscion scierus</i>	<i>Umbrina roncadore</i>	<i>Lutjanus argentiventris</i>	<i>M. furnieri</i>	<i>M. furnieri</i>	<i>M. furnieri</i>
<b>Locality</b>	Mexico (Pacific)	Mexico (P)	Baja California (P)	Mexico (P)	Baja California (P)	Rio de Janeiro Brazil	Argentina (South Atlantic)	Puerto Rico (Caribb. and NA)
<b>Length (µm)</b>	1152-1520 <sup>1,3</sup>	2800-2940 <sup>1</sup>	1470-2730 <sup>1</sup>	1638 <sup>1</sup>	1302-3010 <sup>1</sup>	1319 (938-1800)	1627 (650-2470) <sup>2</sup>	1601 (1140-2410)
<b>Width (µm)</b>	192-208	350-350	185-420	196	154-364	224 (108-474)	320	156.7 (70-230)
<b>Pharynx</b>	Round	oval	-----	-----			oval	oval
<b>Genital spines</b>	10-12	12	12	12	12	7-10	7-11	7-11
<b>Testes</b>	18-20	30-36	20-26	22	22-29		10-14	Around 10
<b>Vagina</b>	One vagina middorsal <sup>1,3</sup>	One vagina middorsal <sup>1,4</sup>	One vagina middorsal <sup>1</sup>	One vagina middorsal <sup>1</sup>	One vagina middorsal <sup>1,4</sup>	One vaginal pore dorsal	No vagina <sup>2</sup> (not observed) <sup>4</sup>	Vagina (not observed)
<b>Small clamps</b>	Share the same peduncle <sup>3</sup>	Each has its own peduncle <sup>4</sup>	-----	-----	Each has its own peduncle <sup>4</sup>	-----	Each has its own peduncle <sup>2,4</sup>	Each has its own peduncle
<b>Lappet position</b>	B/w small clamps <sup>3</sup> B/w large clamps <sup>4</sup>	Not observed	-----	-----	B/w large clamps <sup>4</sup>	-----	B/w large clamps (posteriormost) <sup>2,4</sup>	B/w large clamps (posteriormost)
<b>Eggs (µm)</b>	-----	166□ long by 39□ wide	214 □ long by 56 □ wide	-----	140 long by 49 wide	140 long by 72 wide, filaments 77.4 and 140.4	175 long by 50 wide and prolongation of 75	248 long by 43 wide (with the filaments)

Taxonomic Summary of *Neopteriotrematoides avaginata* Suriano, 1975

**Material examined:** 74 specimens

**Host Examined:** 40 *Micropogonias furnieri* (whitemouth croaker) (Sciaenidae)

**Host localities:** 35 from Guanajibo and Boquilla, Mayagüez, Puerto Rico (Caribbean) and 5 from Vacía Talega, Loíza, Puerto Rico (Atlantic)

**Host infected:** Total 27 (all from Mayagüez)

**Mean Intensity:** 2.7 from the Mayagüez fishes and 0 from the Loíza fishes for an overall intensity of 2.7

**Prevalence:** For the Mayagüez collection 77%, for the Loíza collection 0% for an overall prevalence of 67.5%

**Comparative Material:** Holotype, Helminthological Collection, La Plata Museum, Argentina # P3 and Paratype material (213-2160) of *Pteriotrematoides mexicanum* two slides # 098 “Colección Helmintológica del Instituto de Biología UNAM, Mexico.

***Neopteriotrematoides avaginata* (Suriano, 1975) n. comb.**

**Description:** The body of these worms is 1203  $\mu\text{m}$  long (920-1700  $\mu\text{m}$ ; n=8) by 168.43  $\mu\text{m}$  wide (80- 252  $\mu\text{m}$ ; n=7) (Figure 7a-7b). The **anterior end** has a wide and undulated oral disc bordered by a thin band of glands and one pair of well developed muscular oral suckers inside the oral cavity. The dorsal side of the oral disc extends more anteriorly than the ventral side and has a small bump or protuberance anterior of the glands around the oral disc (Figures 7c-7e). The mouth, observed from the anterior, appears as a heart shape (Figure (7e).

**Digestive System**

The **pharynx** is oval and is located posterior of and between the oral suckers. The **esophagus** is short and the intestinal bifurcation occurs at level of the genital pore, anterior of the cirrus and cirrus bulb. The **intestinal cecae** extend to the posterior end of the body just anterior of the opisthaptor where they rejoin and along with the vitelline follicles enter the opisthaptor and extend dorsally in a single branch almost to the posterior most pair of clamps (Figure 8a).

## Genitalia

A genital atrium was not observed. The **uterus** appears to terminate directly inside the genital pore (Figure 8b, 9a-9b) and passes posteriorly, ventral of the cirrus just beneath the ventral surface. In live worms, the uterus bulges during contractions that move the eggs toward the genital pore. The genital pore is found ventrally at level of intestinal bifurcation and expands greatly when the eggs are laid (see figure 9a-9b)). The cirrus has a muscular base and is armed with three pairs of straight spines. They arise from three different directions and are oriented inward (Figure 8b, 9c). These spines have a wide base, and pointed tips. The posterior pair of spines is the largest of the three and has markedly robust and curved base. This pair of spines is 29.2  $\mu\text{m}$  long (25.2-34.65; n= 7), while the other two pair of spines are smaller. The middle pair is 15.15  $\mu\text{m}$  (14.7-15; n=3) long while the anterior is 11.6  $\mu\text{m}$  long. Posterior of the cirrus is a large cirrus bulb similar to the one observed in both *Pterinotrematoides mexicanum* and *Neopterinotrematoides avaginata*. The testicular field begins posterior of the ovary and continues to the end of the body proper between the intestinal cecae. It was not possible to count the testes because they were obscured by the intestinal cecae and the vitellaria.

The **ovary** is large and occupies almost all of the central part of the body proper. Their anterior is on the ventral side of the body mostly at the edge of intestinal cecum on the right side. From there it bends slightly into the dorsal side and returns to the ventral side near the edge of the left intestinal cecum (Figure 9-d). The vitelline reservoir was obscured by the vitelline follicles. A vagina was not observed in any of the specimens examined.

## Opisthaptor

The opisthaptor is separated from the body proper by a constriction at the level of the joining of the branches of the intestinal cecae and the vitelline ducts. It has eight pedunculated clamps arranged in pairs. The first three pairs have a fire tong shape, are oriented ventrally, and each is on its own peduncle. The last pair of clamps is smaller, and has a structure similar to the small pair of clamps found in *Macrovalvitrema*. This pair of clamps extends posteriorly (Figure 9-e). It was difficult to determine if these two clamps share the same peduncle or if each is on its own peduncle (Figures 9e-9f). There is a large lappet with two pairs of larval hooks 22  $\mu\text{m}$  long (20-23  $\mu\text{m}$ ; n=4) located dorsally between the pair of small clamps and oriented posteriorly (Figure 9g).

## Large Clamps

Each member of the pairs of larger clamps appears as mirror images of the other. These clamps do not appear as highly muscular as those of *Macrovalvitrema*. They are composed of elongated dorsal and ventral valves, having different sclerite arrangements. The **dorsal valve** is larger than the ventral valve (see Figure 10a-10b), is symmetrical and composed of two kinds of sclerites, the **lateral sclerites** and the **median sclerite**. The **lateral sclerites** are interrupted slightly below the level of the strangulation resulting in two segments (Figure 10c). The distal segment is semicircular and distally ends in two slightly recurved tips oriented posteriorly towards the midline of the clamp (Figure 10d), proximally, this segment curves inward toward the midline region ending with a triangular tip which join together ventral of the median sclerite. Together they form a butterfly shaped pad that contacts the surface of the gill. This pad is covered with 7 to 11 rows of short bars radiating from the midline and extending to the edge of the pad. Possibly these bars function in maintaining a grasp on the gill filament (Figure 10e). The proximal



segment of the lateral sclerite is long, thin, and straight; does not reach the level of the strangulation; and terminates distally with the triangular shaped spine (Figure 10f). Basally it articulates with the lateral sclerites of the ventral valve. The **median sclerite** ends distally with two faintly recurved tips which almost touch the tips of the distal ends of lateral sclerites (Figure 10d). Basally this piece is bifurcated in two wide, curved ends which articulate with the holes of the basal sclerite (Figure 10g, 12c).

The **ventral valve** is cardioid shaped, at the distal end and straight and thin at the proximal end and is formed from a single continuous **lateral sclerite** (Figure 11a). At the level of the strangulation both sides of the lateral sclerites fold inward and create asymmetrical accessory sclerites which reach the midzone region of the valve (Figure 11b). The thin part of the lateral sclerites begins between these accessory sclerites (Figure 11c) appearing as a stalk and articulates basally with the sclerites of the dorsal valve. A plate runs almost the extent of the inner side of the valve (Figure 11a). The anterior part of the plate is round 16.14  $\mu\text{m}$  wide (14.7-16.8  $\mu\text{m}$ , n=12) and is covered by a thin muscular wall (Figure 11d). This continues toward the thinner part of the valve (stalk) where a very slight fissure forms in one side, while the other side continues uninterrupted to the base (Figure 11e). This plate articulates with the lateral sclerites prior to the anterior end of the base of the clamp (Figure 11f) Medially the plate does not reach the end of the valve, producing a space, through which the basal part of the median sclerite of the dorsal valve can be seen (Figure 11f). The lateral sclerites of the ventral valve articulate basally with the basal sclerite.

The **basal sclerite** of the large clamps is a folded U-shaped piece with one end covering the external side of the lateral sclerites (Figure 12a) of the ventral valve and the other end reaching the midline zone of the dorsal valve (Figures 12b-12c). It has a hole or a space between these

articulations in which the median sclerite of dorsal valve can be seen (Figure 11f). The covering of the basal valve to the proximal part of the lateral sclerites of the ventral valve appears to be muscular (Figure 12a), while the part that covers the midline area of the dorsal valve appears to be strongly sclerotized (Figure 12b-12c).

### **Small clamps**

The pair of small clamps is oriented posteriorly and has a similar arrangement of sclerites as *Macrovalvitrema* (Figure 9e), but without a strangulation in the middle of the clamp (Figure 13a). The dorsal valve has an interrupted lateral sclerite at both the distal end and at the middle of the clamp. The proximal segment of the lateral sclerite terminates with two curved tips, while the distal segment ends at the middle of the clamps terminating with a slight curve oriented toward the midline of the clamp. Basally these pieces articulate with the basal sclerite. The distal end of the median sclerite occurs slightly above the lateral sclerites and basally ends in two curved tips which articulate with the basal sclerite (Figure 13b).

The ventral valve has a continuous lateral sclerite with no interruption in the distal or middle of the clamp (Figure 13a). Basally the opposite sides of the lateral sclerite fold inward creating wide, curved accessory sclerites oriented toward the midline of the clamp. They are unequal with one longer and more curved at tip than the other. This valve also has the characteristic plate of the large clamps that articulates basally with the lateral sclerites, but medially does not reach the base of the clamps. A thin fissure is visible in one of the edges forms, while the opposite side (with the longer curved end) is continuous ending at the base of the lateral sclerite (Figure 13a).

The basal sclerite of these clamps is identical to the ones in *Macrovalvitrema* clamps (as described for *M. argentinensis* in this study).

### **Eggs and embryonic development**

The eggs are 248.3  $\mu\text{m}$  long (including the filaments) (245.5-250  $\mu\text{m}$ ; n=3) by 44  $\mu\text{m}$  wide (42 - 48  $\mu\text{m}$ ; n= 3 and have one filament at each pole. Although most of the eggs have two elongated, straight filaments, a few have a curved tip at one end, while others have a very short filament at one pole. Upon oviposition, the inside of the fertilized eggs, are almost completely filled with vitelline cells, except for a small circular space in the center (Figure 13c). Unfertilized eggs are easy to differentiate because they are completely filled with vitelline cells. In fertilized eggs, migration of the vitelline cells toward the opposite poles was observed three days after the oviposition, leaving the center of the eggs almost empty (Figure 13d). A core in the center of the eggs and the reduction of the vitelline cells at the poles were observed on the sixth day (Figure 13e) as well as movement of the developing larva in the egg. At seventh day the unhatched larvae were observed well defined as well as their hooks arranged circularly in the posterior part of the body (Figure 13f). At the 9<sup>th</sup> day the larvae began to hatch.

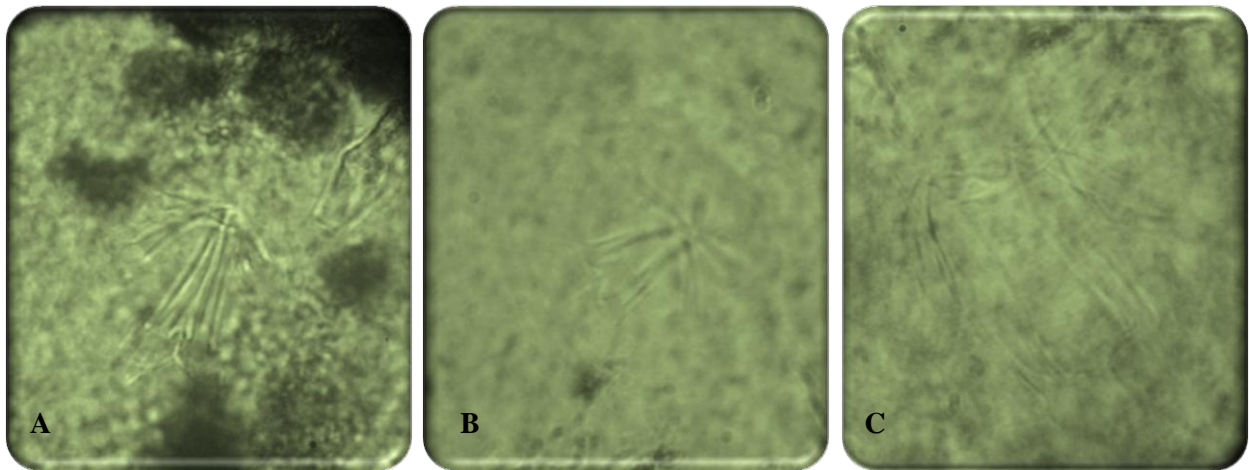
### **Description of the larvae**

The larva or the oncomiracidium has no eyes spots, is 133.35  $\mu\text{m}$  long by 35.18 $\mu\text{m}$  wide (Figure 13 g), and has cilia covering the entire body except the sides of the attachment organ and the tip of posterior end. The digestive apparatus (Figure 13f-g) is very distinct with a thin anterior end, a spherical pharynx 15.75  $\mu\text{m}$  in diameter, and a sac-shaped intestine at the center of the body extending posteriorly to the beginning of the attachment organ. The attachment organ is separated

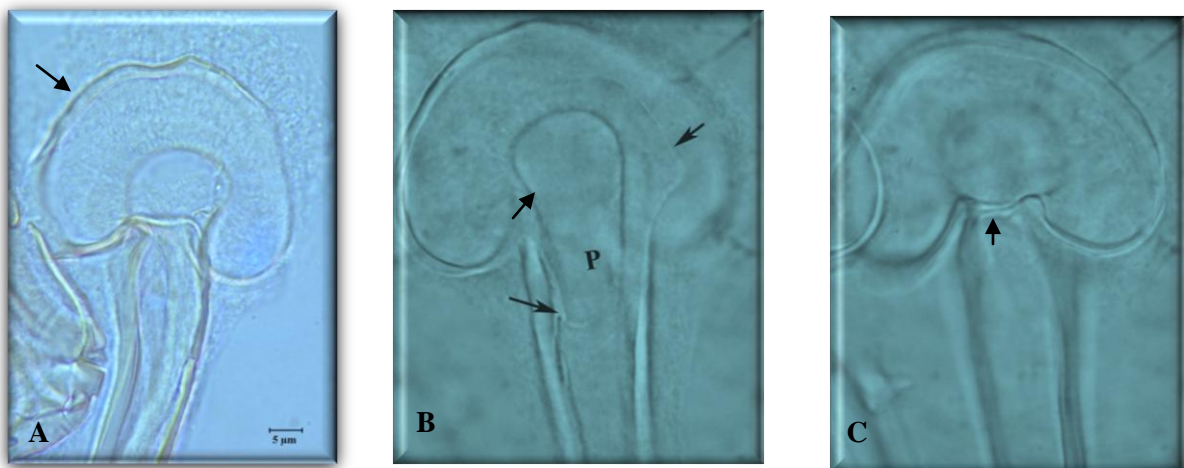
from the rest of the body by a slight constriction (Figure 13g) and ends in a small cone-shaped posterior end that is armed with 12 hooks. . The anterior part of the body contracts and pushes toward the anterior pole of the egg, until the egg splits at level of the operculum, which then widens and the larvae begins to leave. Only one larva was observed in the process of hatching and it died before completely leaving the egg. The rest of the larvae were observed inside of the eggs, or after they hatched from the eggs when they were almost dead.

### Remarks

The general morphology of these worms agrees with the description of *Neopterinotrematoides avaginata* (Suriano, 1975) including the lack of a vagina (despite studying specimens mounted in a lateral position). They also have the same host and the same male genitalia as *N. avaginata*. This species is very similar to *Pterinotrematoides mexicanum* including the number of cirrus spines, but differs in the size of these spines with *P. mexicanum* having more robust (thicker) spines than *N. avaginata* which has more delicate and small spines (Figure I-1). Also in *N. avaginata* the shape of the distal part of ventral valve is cardioid-shaped, the plate is extended to the distal segment of the ventral valve and is surrounded by a muscular wall and has accessory sclerites at strangulation level (Figure I-2), while in *P. mexicanum* the distal part of the ventral valve is oval or semicircular, the plate is limited to little bit anterior to the strangulation level and does not have the accessory sclerite at strangulation level. None of these differences were identified by Suriano (1975), which establishes the new genus based only in the absence of the vagina. Table I-3 shows some details of all the material identified as *P. mexicanum* by Caballero y C. and Bravo-Hollis (1982) from the Pacific coast of Mexico and California, the material identified as *P. mexicanum* by Kohn et al. (1989), the material of *N. avaginata* studied by Suriano (1975) from Argentina and the material of *N. avaginata* collected in this study from Puerto Rico.



**Figure II-1** Comparison of the cirral spines of *Neopteriotrematoides avaginata* Suriano, 1975 and *P. mexicanum* Caballero y C. and Bravo-Hollis, 1955. **A.** *N. avaginata* (Caribbean); **B.** *N. avaginata* (Argentina); **C.** *P. mexicanum* (Mexico). \*This pictures were taken with the same magnification.



**Figure II-2.** Generic Differences of the large clamps of *Neopteriotrematoides avaginata* Suriano, 1975. **A.** cardioid shape of the distal part of the ventral valve; **B.** extend of the plate to the distal part of the ventral valve and the muscular wall that is around it. **C.** presence of the accessory sclerite at strangulation level.

Caballero y C. and Bravo-Hollis (1982) described *P. mexicanum* originally from the sciaenid *Micropogonias ectenes*, later Bravo-Hollis (1982) reported material collected from the sciaenids *Ophioscion scierus*, *Umbrina roncadore* and *M. megalops* as well as material collected from the

lutjanid *Lutjanus argentiventris*, as the same species. They had minor differences mostly in measurements, in the number of testes and the number of genital spines, but Bravo-Hollis (1982) did not considered these differences sufficient to distinguish a new species. (Table I-3).

The apparent absence of a vagina in the specimens studied by Suriano and the ones collected in this study is not conclusive to erect a new genus. But the size of the male genital apparatus with respect their body size and the differences of the large clamps (shape of the distal segment of ventral valve, extend of the plate and the presence of accessory sclerite) are considered sufficient to distinguish them from *P. mexicanum*. This finding represents new locality record for *Neopteriotrematoides avaginata* Suriano 1975, which previously was reported from Mar del Plata, Argentina. There are some misinterpretations and details not observed by Suriano (1975), Caballero y C and Bravo-Hollis (1955) and Bravo-Hollis (1982).

**First:** The observations made during the oviposition of the eggs, demonstrates that the uterus ends directly in the genital pore, which is located ventrally at the level of the intestinal bifurcation. The uterus runs along the body just under the ventral surface, and the cirrus runs dorsal to the uterus and is armed with 3 pairs of spines, which Suriano (1975) referred as atrial spines.

**Second:** Suriano (1975) did not note the difference in size and shape of the male genitalia.

**Third:** Not mentioned in either of the descriptions of *N. avaginata* and *P. mexicanum* is that the sclerite composition is a modification of the sclerites found in *Macrovalvitrema* species.

**Fourth:** The detail of the lateral sclerites of the dorsal valve of the small clamps was not described by Suriano (1975) in her original description of *N. avaginata* or shown in the plates. The drawings of *P. mexicanum* by Caballero y C. and Bravo-Hollis (1955) show the division of

the lateral sclerites, as well as the plate of the ventral valve (but not the fissure), although they did not mention or describe it in the text.

Table II-3 Comparison of the two species of the genus *Pterinotrematoides* Caballero y C. and Bravo-Hollis (1955)

	<i>Pterinotrematoides mexicanum</i> Bravo-Hollis (1982)					<i>P. mexicanum</i> Kohn et al. (1989)	<i>Neopterinotrematoides avaginata</i> Suriano 1975	<i>N. avaginata</i> Suriano 1975 New Material
	Specimen Original	Specimen 2	Specimen 3	Specimen 4	Specimen 5			
Host	<i>Micropogonias ectenes</i>	<i>M. megalops</i>	<i>Ophioscion scierus</i>	<i>Umbrina roncador</i>	<i>Lutjanus argentiventris</i>	<i>M. furnieri</i>	<i>M. furnieri</i>	<i>M. furnieri</i>
Locality	Mexico (Pacific)	Mexico (P)	Baja California (P)	Mexico P)	Baja California (P)	Rio de Janeiro Brazil	Argentina (South Atlantic)	Puerto Rico (Caribb. and NA)
Length (µm)	1040-1088 <sup>3</sup>	1608-2940 <sup>1</sup>	1050-1316 <sup>1</sup>	1092-1120 <sup>1</sup>	1330-1694 <sup>1</sup>	1509 <sup>5</sup> (900-2272)	1439 <sup>2</sup> (1525-2106)	1203 (920- 1700) <sup>4</sup>
Width (µm)	160-176 <sup>3</sup>	378-462 <sup>1</sup>	168 <sup>1</sup>	154-182 <sup>1</sup>	210-238 <sup>1</sup>	224 <sup>5</sup> (108-474)	297.5 <sup>2</sup> (208-429)	168.43 <sup>4</sup> (80- 252)
Pharynx	Oval <sup>3</sup>	Not stated	Not stated	Not stated	Not stated	Oval <sup>5</sup>	Oval <sup>2</sup>	Oval <sup>4</sup>
Largest cirral spine	30-34□m <sup>3</sup> 43.6□m <sup>4</sup> (42-45.9)	Not stated	Not stated	Not stated	Not stated	(15-24)□m <sup>5</sup>	25□m <sup>2</sup> 29.92□m <sup>4</sup>	29.18 <sup>4</sup> (25.20-34.65)
Testes	10-16 <sup>3</sup>	19-21 <sup>1</sup>	14-15 <sup>1</sup>	Not stated	16 <sup>1</sup>	Not stated	Not stated	Not stated
Vagina	One dorsal <sup>3</sup> Two lateral <sup>1</sup>	Not stated	Not stated	Not st ed	Not stated	Two lateral <sup>5</sup>	Not observed	Not observed <sup>4</sup>
Eggs (µm)	150 long by 60 wide; filaments (112 and 56) <sup>3</sup>	Not stated	Not stated	Not stated	Not stated	146-161 long with two filaments ( 36- 48 and 124) <sup>5</sup>	250 with filaments (estimated from picture) <sup>4</sup>	248.3 long <sup>4</sup> (245.5-250) with filaments

\* The information of the genera *Macrovalvitrematoides*, *Pseudotagia* and *Papillopseudotagia* is not included in the table because they have well marked differences with the new collected material.

<sup>φ</sup> This refers to the type material described by Caballero y C. and Bravo-Hollis, 1955 and all the information about the number of genital spines and testes were recovered from Bravo-Hollis, 1982.

The information of this table were recovered from: <sup>1</sup>Bravo-Hollis (1982), <sup>2</sup>Suriano (1975), <sup>3</sup>Caballero y C. and Bravo-Hollis (1955), <sup>4</sup>personal observation and Kohn et al (1989)



Taxonomic Summary of Genus N., n. sp.

Synonym: *Pterinotrematoides mexicanum* of Fuentes et al. (2009) from Venezuela

**Material examined:** 25 specimens

**Hosts examined:** 24 *Archosargus rhomboidalis* (sea bream) (Sciaenidae)

**Host Locality:** El Combate, CaboRojo (Caribbean

**Host infected:** 13 infected hosts

**Mean Intensity:** 25 parasites from 13 infected hosts= 1.92; **Prevalence:** 54%

Additional Specimens: 3 (previously identified as *P. mexicanum*) of Williams, E. and L. Bunkley-Williams collection from the same host from La Parguera, Lajas.

### Genus New A species new

**Description** (Based on 18 specimens, five measured). The body of this worm agrees with the diagnosis of the family Macrovalvitrematidae. The body, total length 2420  $\mu\text{m}$  long (2040-2600  $\mu\text{m}$ ; N=5) and 227  $\mu\text{m}$  wide (140-360; n=5) at level of the ovary, is characterized by an extremely wide anterior end, long body proper extended with a peduncle and relatively small opisthaptor (Figures 14a,g).

#### Anterior end:

The anterior end of this worm is similar but distinctly longer and wider than the rest of macrovalvitrematid species, measuring 260  $\mu\text{m}$  long by 486  $\mu\text{m}$  wide in its widest part and 324 at its narrowest part (Figures 14b-c). It has a pair of rectangular oral suckers in the oral cavity that extend to the rims of the oral disc (Figure 14d), and are 158  $\mu\text{m}$  long (152-168  $\mu\text{m}$ ; n=6). These suckers are strongly muscular at the base as well as in the distal edge and are also slightly sclerotized (Figures 14d-e). The oral disc has an irregular rectangular shape with the dorsal side extending anterior of the ventral side being able to cover it when it is closed (Figure 14f). The anterior edges of the oral disc are undulated or scalloped (Figure 14b-c).

#### Digestive System:

These worms lack a **pharynx**, the food passes dorsally to the **esophagus** that leads to the intestinal cecae (Figure 14f). The **intestinal bifurcation** occurs at the level of genital atrium. The intestinal cecae and

vitelline follicles extend to the posterior end of the body proper just anterior of the peduncle where the cecae rejoin (Figure 14g) and along with the vitelline follicles enter the opisthaptor and extend dorsally in a single branch almost to the posterior most pair of clamps (Figure 15a).

### **Reproductive System**

The **copulatory organ** has a round muscular base 29  $\mu\text{m}$  of diameter (27-31  $\mu\text{m}$ ; n= 4) (Figure 15b and 15e) and is posterior to the intestinal bifurcation. Its armature consists of 5 spines with one central spine much larger than the other two pairs. The large spine is oriented from posterior to the anterior and has a broad base 19  $\mu\text{m}$  wide embedded in the muscular wall (Figure 15e). The two other two pairs are oriented from the posterolateral sides toward the anterior. The base of these two pairs of spines is also wider than the distal end (Figure 15c). Nine to ten oval **testicles** occupy the posterior of the body proper, anterior of the rejoining of the intestinal branches. The uterus runs close to the ventral surface and ends in the genital atrium at the level of the intestinal bifurcation. The genital aperture is at the level of the genital corona. During the oviposition the uterus can create several bulges along the ventral surface. The single egg observed had only one filament (Figure 19c). The egg measures 230  $\mu\text{m}$  long including the filament by 50  $\mu\text{m}$  wide. This egg did not develop.

This worm has two **vaginae** located ventrolaterally (one on each side) at the level of the vitelline reservoir (Figure 16a). The vaginal apertures are characterized by several folds and a slight invagination and the vaginal openings are covered by thin lids (Figure 16b). The **ovary** is located in the middle of the body proper anterior to the testes. The anterior is located ventrally close to the intestinal branch of the right side of the body, passing posteriorly, it bends toward the dorsal side of the body and widens as it returns to the ventral side oriented toward the left side of the body; then bends again and narrows before it reaches the left intestinal branch on the dorsal side of the body (Figure 16a).

## Opisthaptor

The **opisthaptor** is separated from the body proper by a short peduncle (reduction of the width) and is approximately the same or less width as the oral disc (see Figure 14g). It has four pairs of clamps, the anterior three pairs are fire tong shaped each on its own peduncle and are arranged in a row with a ventral orientation (Figure 17a). The posterior most pair of clamps is small, shares the same peduncle and is similar in structure to the small clamps of *Nicolasia canosorum* Suriano, 1975 and *Pseudohargisia cortesi* Payne, 1987. A lappet was observed in some of the specimens between the posterior most clamps (the smallest pair). Only one pair of hooks was observed on it measuring 16.4  $\mu\text{m}$  long (16-16.8  $\mu\text{m}$ ; n=2) (Figures 17b-c).

## Large Clamps

The three large pairs of clamps are highly sclerotized and composed of two dissimilar valves (dorsal and ventral) (Figure 18a, 18d). The **dorsal valve** 146  $\mu\text{m}$  long (142-149  $\mu\text{m}$ ; n= 8) is symmetrical and slightly larger than the ventral valve. It is composed of two lateral sclerites and a median sclerite. The **lateral sclerites** are interrupted below the strangulation of the valve resulting into two segments (distal and proximal) (Figure 18b). The distal segments are wide 86  $\mu\text{m}$  (83-97  $\mu\text{m}$ ; n= 7) and have a flattened semicircular shape (Figure 18b). The distal portions of this segment turn proximally as they approach each other at the midline of the valve forming a butterfly shape (Figure 18a) While at the level of the strangulation both sides slightly curved toward the midline zone, reduce in width, then turn abruptly proximally to form a hanging sclerite 30  $\mu\text{m}$  long (28-31  $\mu\text{m}$ ; n=5) (Figure 18b). The lateral sides of the distal lateral sclerites have approximately seven short and flat spines 4  $\mu\text{m}$  long (Figure 18b and 18c). This valve also has faintly visible rows of bars similar to those observed in *Pterinotrematoides*, *Nicolasia*, and *Pseudohargisia* (Figure 18c).

The proximal segments of the lateral sclerites are short and thin, approximately 43  $\mu\text{m}$  long (37-49  $\mu\text{m}$ ;  $n=6$ ), are less than half the length of the median sclerite, not extending to the level of the strangulation or the hanging sclerite (Figure 18b). Basally they articulate with the anterior side of the basal sclerite (Figure 18g). The **median sclerite** 119  $\mu\text{m}$  long (117-121  $\mu\text{m}$ ;  $n=6$ ) bifurcates at both ends, distally it has one pair of slightly curved tips that extend to the center of the butterfly-shaped area, and basally it widens and becomes a pair of large curved tips that articulate with two holes in the basal sclerite (Figure 18b, 18d and 18g). The inner surface of this valve has an accessory sclerite, approximately 24  $\mu\text{m}$  long that arises from the termination of the distal end of the median sclerite, and extends almost to the edge of the valve (Figure 18d-e).

The **ventral valve** 133  $\mu\text{m}$  long (129-135  $\mu\text{m}$ ;  $n=4$ ) is slightly shorter than the dorsal valve. It is composed of a continuous lateral sclerite and the basal sclerite (Figure 18d). The **lateral sclerite** is wide 68  $\mu\text{m}$  (61-74  $\mu\text{m}$ ;  $n=7$ ) and semicircular anteriorly, narrows at level of the strangulation and becomes an elongated and slender with a similar width as the median sclerite of the dorsal valve (Figure 18d). This valve does not possess any accessory sclerites, but has around five tiny, flattened spines in the edges of the widest part and three to five along middle area of the slender part of this sclerite (Figure 18a). The plate found along this valve as in other macrovalvitrematids (e.g., *Macrovalvitrema sinaloense*, *M. argentinensis*, *Pterinotrematoides mexicanum*, and *P. avaginata*) was not observed in detail in the large clamps. However in a side view of the slender part of the lateral sclerite a groove is observed, which seems to belong to this plate. Also this valve reaches the basal sclerite laterally and not medially as occurs with the plate of other macrovalvitrematids studied (Figure 18f). In addition, one side of this lateral sclerite is continuous with the basal sclerite while the other side articulates with it (Figure 18f). The groove observed in the side view of the lateral sclerite is in the side that articulates with the basal sclerite and not in the side that is fused with the basal sclerite.

The slender part of the clamps of these worms is narrower (approximately 18  $\mu\text{m}$ ) than those in the other macrovalvitrematids studied. The same is true of their **basal sclerite** 20  $\mu\text{m}$  wide (16-23  $\mu\text{m}$ ; n=5), which are clearly part of, or fused with, the lateral sclerite of the ventral valve and oriented toward the dorsal valve with an U-shape end (Figure 18f). It has a pair of holes in which the median sclerite of the dorsal valve articulates.

### **Small pair of clamps**

The posterior most pair of clamps is small with similar sclerite organization compared to the small clamps of *Nicolasia canosorum* and *Pseudohargisia cortesi* and both clamps share one enlarged peduncle which is oriented posteriorly or dorsally (Figure 17a-c). Their valves do not have a strangulation in the middle and are asymmetrical, appearing as mirror images of each other.

The **dorsal valve** (Figure 19a) 64  $\mu\text{m}$  long (61-65  $\mu\text{m}$ ; n=7) by 43  $\mu\text{m}$  wide (41-45  $\mu\text{m}$ ; n=5) is also composed of the lateral sclerites and the median sclerite. The lateral sclerites of the dorsal valve are discontinuous at both sides, creating two segments (distal and proximal). The distal segments join at the midline. The proximal segments are straight and articulate basally with basal sclerite. The median sclerite 51  $\mu\text{m}$  long (50-52  $\mu\text{m}$ ; n=6) has two bifurcated ends. The distal end has slightly curved tips which exceed the height of the ventral valve. The proximal end has two wider but small curved tips which articulate with the basal sclerite.

The **ventral valve** (Figure 19b) 50  $\mu\text{m}$  long (49-50  $\mu\text{m}$ ; n=6) by 39  $\mu\text{m}$  wide (37-41  $\mu\text{m}$ ; n= 6) is composed of the continuous lateral sclerite and the plate which occupies one lateral and proximal side of the valve. It is this valve that exhibits the mirror image of the clamp pair. This lateral sclerite join distally in a round end , but proximally both sides fold basally to produce an accessory sclerite oriented toward the center of the clamp (Figure 19a). These accessory sclerites are different, with the ones on the outer

side of the clamp larger, 12  $\mu\text{m}$  (11-13  $\mu\text{m}$ ; n=5) than the ones in the inner side (side that is close to the other clamp). The plate in these clamps is reduced and limited to one lateral side of the valve, but articulates with both lateral sclerites, but medially the plate does not reach or articulate with the basal sclerite. The **basal sclerite** has a U-shape as well as two holes in which the median sclerite articulate, as do the small clamps of *Nicolasia* and *Pseudohargisia*.

### Remarks

These worms agreed with the most distinctive characters of the family Macrovalvitrematidae (goblet-shaped anterior end, armed genitalia, and opisthaptor composed by eight pedunculated clamps).

### Generic level characters and comparison to the other genera

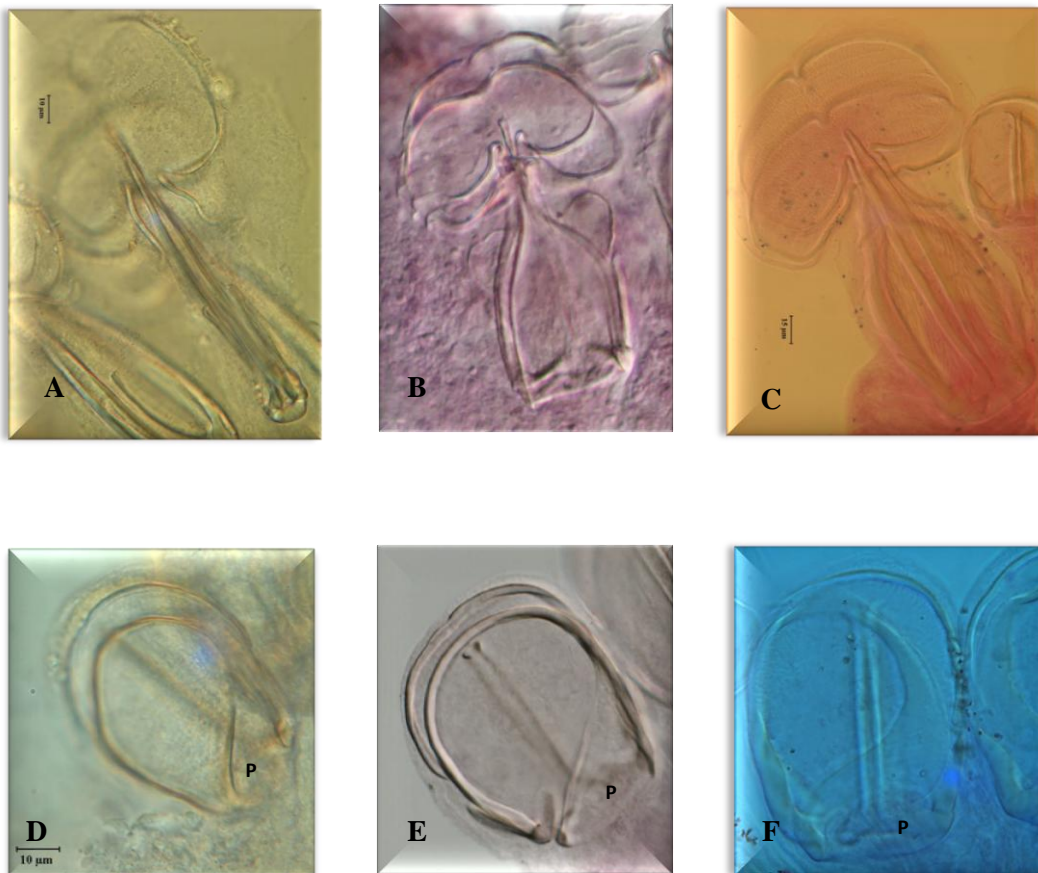
This worm differs from all other genera in the family Macrovalvitrematidae by having the anterior end and the oral disc much larger and wider than the width of the body proper, by having a pair of rectangular and sclerotized-bordered oral suckers, by lacking a pharynx and having several short spines along the distal segment of the lateral sclerites of dorsal valve as well along all the lateral sclerites of ventral valve. All other macrovalvitrematids have the oral disc the same or smaller width than the body proper, oval and muscular oral suckers and small and round pharynx present posterior of and between the oral suckers. In addition the anterior end of the body has a similar width as the opisthaptor, while in the other macrovalvitrematids the opisthaptor is distinctly wider than the anterior end.

### Species level characters and comparison with other species

#### A. Comparison with *Pseudohargisia* and *Nicolasia*:

This species most closely resembles *Pseudohargisia cortesi* and *Nicolasia canosorum* in the absence of glands, the presence of hanging sclerites at the level of the strangulation and in the shape of the pair of

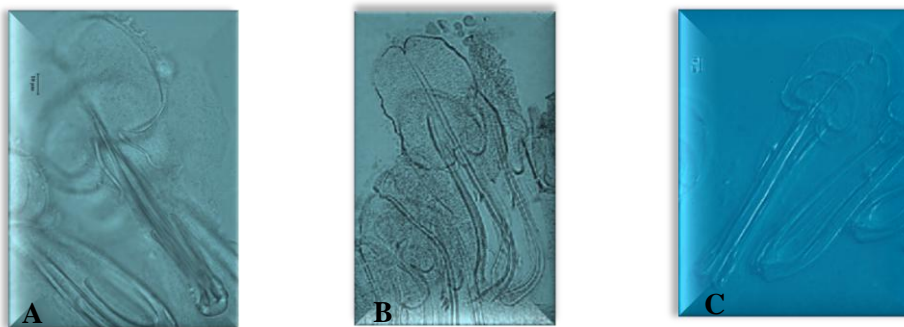
small clamps. These three are the only macrovalvitrematids which do not have distinctive glands around the oral disc and the only ones with the small pair of clamps with the plate located in one of the lateral and proximal sides of the ventral valve. This position of the plate is also observed in the large clamps of *Papillopseudotagia hubbsi*. Also the presence of hanging sclerites as an extension of the distal segment of the lateral sclerite is only observed in these three species (*A. cortesi*, *N. canosorum* and, this new species). But the hanging sclerite shape of this new species is similar to those in *Pseudohargisia cortesi*. *N. canosorum*, in addition the hanging sclerite has an accessory that joins it to the median sclerite. *Pseudohargisia cortesi* has an additional accessory sclerite posterior to the hanging sclerite not found in the new species.



**Figure II-3.** Comparison of large (A, B and C) and small (D, E and F) clamps of the New Genus (A and D); *Pseudohargisia cortesi* (B and E) and *Nicolasia canosorum* (C and F).

### B. Comparison with *Pterinotrematoides* and *Neopterinotrematoides*

This new Genus and species also closely resembles to *Pterinotrematoides mexicanum* and *Neopterinotrematoides avaginata* in the shape of the distal segments of the dorsal valve, the arrangement of copulatory spines, and in the basal sclerite arrangement. These three species are the only of macrovalvitrematids that share a butterfly-shaped distal segment of the lateral sclerites of the dorsal valve. These three species are the only macrovalvitrematids that do not have radially arranged spines of equal morphology in the male copulatory organ. *Pterinotrematoides mexicanum* and *N. avaginata* have three pair of spines oriented from different positions arranged bilaterally symmetrical, with the posterior pair the larger. The new genus has five spines, one large in the center and two smaller pairs arranged bilaterally symmetrical oriented from a posterolateral position. In the large clamps of the new genus, the width of the proximal segment of the ventral valve is reduced to almost the same width as the median sclerite, as occurs in the genera *Pterinotrematoides* and *Neopterinotrematoides*, but not in the other genera and species in this family. The same occurs in the three species with the basal sclerite, which also have a U-shape in both ends. The only difference is that in *P. mexicanum* and *N. avaginata* this articulates with the lateral sclerites of ventral valve covering it, while in this new genus and species the basal sclerite is fused with the lateral sclerite of ventral valve.



**Figure II-4** Comparison of clamps New Genus (A); *Pterinotrematoides mexicanum* (B); *Neopterinotrematoides avaginata* (C)



### Other similarities:

In addition this species shares similarities with both species pairs (*Pseudohargisia* and *Nicolasia*) and (*Pterinotrematoides* and *Neopterinotrematoides*) in the position of the small clamps, the strangulation at midline longitudinal level in the large clamps, the absence of strangulation in the small clamps and in the presence of bars in the distal segment of dorsal valve.

### Previous reports

Fuentes, et al. (2009) reported one macrovalvitrematid from *Archosargus rhomboidalis* from Isla de Margarita, Venezuela, which in addition to the host and locality (Caribbean) the drawing seemed to indicate that it is the same species as the ones reported in this study. The drawing agrees with these specimens in the size of the anterior end, oral suckers and in the absence of the pharynx. However, they identified their worms as *Pterinotrematoides mexicanum* and comment that they agree in almost all the characters previously identified by other authors. Nevertheless, several conspicuous characters separate these worms from the rest of the macrovalvitrematids. These have been discussed above. The only difference with the description of Fuentes et al. (2009) is that they only observed one vagina, while in the specimens examined in this study two vaginae were clearly observed.

Taxonomic Summary of *Pseudotagia* sp. n.

**Material examined:** 49 specimens

**Hosts Examined:** 14 *Pomadasys corvinaeformis* (roughneck grunt) (Sciaenidae)

**Host Localities:** 10 from Guanajibo and Guayabo, Mayagüez, Puerto Rico (Caribbean) and 4 from Loíza, Puerto Rico (Atlantic).

**Hosts Infected:** Total of 13 infected (10 hosts from Mayagüez with 39 parasites and 3 from Loíza with 10 parasites).

**Mean Intensity:** 3.9 for the Mayagüez fish and 3.3 for the Loíza fish for a total of 49 parasites in 13 hosts infected = 3.77.

**Prevalence:** 100% for Mayaguez and 75% for Loiza for an overall prevalence of 93%.

***Pseudotagia* sp. n**

**Description:** (based in 20 specimens, 11 measured). The body of these worms (Figure 20a-b) is 1684  $\mu\text{m}$  long (980-2480  $\mu\text{m}$ ; n=11) by 294  $\mu\text{m}$  wide (130-420  $\mu\text{m}$ ; n=11). The anterior end has a wide oral disc approximately 255  $\mu\text{m}$  in diameter bordered by a thin band of glands, and one pair of oval oral suckers 114  $\mu\text{m}$  long (113-115  $\mu\text{m}$ ; n=3) by 92  $\mu\text{m}$  wide (79-100  $\mu\text{m}$ ; n=3) inside the oral cavity (Figures 21a-b).

**Digestive System**

The **pharynx** is oval [44  $\mu\text{m}$  long (40-46  $\mu\text{m}$ ; n= 5) by 37  $\mu\text{m}$  wide (34-39  $\mu\text{m}$ ; n=5)] and located dorsal of and between the oral suckers. The oral suckers have a muscular base, which extends to the lateral edge. The dorsal side of the oral disc extends slightly more anterior than the ventral side. The **esophagus** is relatively short and the **intestinal bifurcation** occurs at the level of the genital atrium, just posterior of the genital corona (Figure 21a). The intestinal cecae extend to the posterior end of the body just anterior of the opisthaptor where they rejoin and along with the vitelline follicles, enter the opisthaptor, and extend dorsally in a single branch almost to the posterior most pair of clamps (Figure 20a-b, 21e).

**Genitalia**

The **genital corona** of the male copulatory organ [43.4  $\mu\text{m}$  in diameter (40-52.5  $\mu\text{m}$ ; n=7)] located close to the anterior end of the body, is spherical, with a muscular base and is armed with 7-8 bifid or bifurcated spines 12.4  $\mu\text{m}$  long (Figure 21a, 21c). The distal ends of these spines are pointed and oriented toward the center of the aperture; while the bases are wider and embedded into the muscular base. In one worm 10-12 round postovarian testes, were observed occupying the posterior portion of the body proper.

The **ovary** is large and located in the medium intercecal zone, anteriorly starting close to the intestinal cecum on the right side, passing posteriorly to the center of the body where it turns to the dorsal side of the body, and then back again to the ventral side close to the left intestinal cecum and ends at the central area of the body proper (Figure 20b). Two specimens had eggs in the uterus. Each egg had one round pole and one fusiform pole 175  $\mu\text{m}$  long (150-200  $\mu\text{m}$ ; n=2) (without the filament) by 68  $\mu\text{m}$  wide (68  $\mu\text{m}$ ; n=2) (Figure 24b). The vitelline reservoir, the genitointestinal canal and the Mehlis gland were not observed. In one specimen something that could be the dorsolateral vaginae (one at each side), were observed, but they could not be clearly seen.

### **Opisthaptor**

The opisthaptor is composed of eight small clamps, each on its own peduncle, arranged symmetrically in pairs (Figure 21d-e.). The two central pairs are larger [84 $\mu\text{m}$  long (76-91  $\mu\text{m}$ ; n=7) and 83  $\mu\text{m}$  long (72-88  $\mu\text{m}$ ; n= 7)] than the two outer pairs. The outer pairs, are similar in size with the anterior most pair slightly larger [73  $\mu\text{m}$  long (60-80  $\mu\text{m}$ ; n=7)] than the posterior most pair, [70  $\mu\text{m}$  long (70-77  $\mu\text{m}$ ; n=7)]. In most worms a lappet was observed at the end of the opisthaptor between the last pair of clamps (Figure 22a-b). It had two pairs of hooks, the central pair [16.7  $\mu\text{m}$  long (15.8-17.85  $\mu\text{m}$ ; n=5)] while in the lateral position a domus [10.2  $\mu\text{m}$  long (9.45-10.5  $\mu\text{m}$ ; n=5)] was observed instead of lateral hooks.

The clamps have two plate-like valves (dorsal and ventral) and a wide basal sclerite (Figure 22c, 22d). The **dorsal valve** is slightly larger with a more complex structure than the ventral valve. The dorsal valve is composed of two lateral sclerites and one median sclerite with a reinforcement accessory (Figures 23a, 24a). The lateral sclerites are very thin, smooth and continuous, but widen as they reach the distal extent of the valve, where the tips end in a wide curve oriented toward the interior of the clamp (Figure 23a). The lateral sclerites are covered by a thin plate or lamina that, although extending toward the center of the

valve, does not reach the median sclerite or their reinforcement accessory (Figure 23b). These plates or laminae are wide distally and remain wide along almost the total extent of the valve, but proximally turn thinner and articulate with the basal sclerite next to the articulation of the lateral sclerite (Figures 23a, 24a). The **median sclerite**, is bifurcated at both ends, is thinner at the distal end, and the proximal end is wide and articulates with the dorsal side of the basal sclerite (Figures 23a, 24a). The reinforcement accessory of the median sclerite borders the whole length of the valve and distally is thin and close to the tips of the median sclerite and proximally opens into a pair of curved ends which wrap around posterolaterally to articulate with the accessory sclerite of the ventral valve (Figures 23a, 24a) at the side of the valve. This piece turns straight again basally at the level of the basal end of the median sclerite and articulates with the basal sclerite (Figures 23a, 24a).

A pair of thin sclerites is articulated to the dorsal side of the basal sclerite and is oriented toward the ventral valve to reinforce the sides of this valve. The **ventral valve** is composed of the lateral sclerites and a small plate. The lateral sclerites are thin and are fused distally and articulate proximally with the basal sclerite. The plate is located at proximal part of the valve and articulates with the lateral sclerites anterior of the basal sclerite, then folds around to the other side in a small pair of accessory sclerite (different on each side). The plate does not articulate medially with the basal sclerite.

The **basal sclerite** is very wide and ring shaped. The dorsal side is U-shaped and a small portion has several articulations with the sclerites of the dorsal valve. The ventral side is straight and only articulates with the lateral sclerites of the ventral valve. The large central area of this sclerite is open and free of articulation.

**Table II-4 Comparison of the species of *Pseudotagia* Yamaguti 1963**

Species	<i>Pseudotagia cupida</i>	<i>Pseudotagia clathratus</i>	<i>Pseudotagia rubri</i>			<i>Pseudotagia</i> n. sp.
Author	(Hargis 1956) Yamaguti 1963	Crane 1972 (Holotype and paratype)	Luque et al. 1993 (Paratype)	Kohn et al. (1984)	Kohn et al. (1992)	New collection
Host Family	Haemulidae	Serranidae	Haemulidae	Haemulidae	Serranidae:	Haemulidae
Host species	<i>Orthopristis chrysoptera</i>	<i>Paralabrax clathratus</i>	<i>Orthopristis ruber</i>	<i>Haemulon sciurus</i>	<i>Diplectrum</i> sp.	<i>Pomadasy corvinaeformis</i>
Locality	Alligator Harbor, Franklin Co. FL	Pacific Ocean, San Diego and La Jolla, California	Itacurucá, Sepetiba Bay, Rio de Janeiro State, Brazil	Ilha do Governador Rio de Janeiro State, Brazil	Ilha do Governador Rio de Janeiro State, Brazil	Mayagüez (Caribbean) and Loíza (Atlantic) Puerto Rico
Length (µm)	1400 (1200-1500)	900 ( 680-1090)	1530 (1240-1830)	1470 ( 1280-1800)	1320 long	1684 (980-2480)
Width (µm)	213 (172-268)	240 (110-300)	302.5 (220-366)	270 (230-300)	180 wide	294 (130-420)
Oral cavity	Not stated	Not stated	Subterminal	Terminal	Not stated	Subterminal
Oral suckers (µm)	Not stated	110 long (70-140) by 70 wide (50-90)	62 in diameter (51-73)	Not stated	60 in diameter	114 long (113-115) by 92 wide (79-100)
Pharynx (µm)	Ovoid, 47 long (34-61) by 37 wide (31-43)	Ovoid, 40 long (30-60) by 30 wide (20-40)	Ovoid, 60.5 long (51-69.5) by 46.4 wide (38.4-55)	Ovoid, 53 long (48-66) by 45 wide (40-49)	??? 36 long by 46 wide	Ovoid 44 long (40-46) by 37 (34-39)
Esophagus	Very short	Not stated	Not stated	Not stated	Not stated	Short
Intestinal bifurcation	Anterior to the genital corona	Anterior to genital atrium	At genital atrium level	Not observed because vitellaria	Not stated	At level of genital atrium, below the genital corona
Intestine end	Posterior end not observed	Not confluent	Not confluent	Not observed	Not stated	Confluent
Vitellaria	Between vagina and opisthaptor	Between genital atrium and opisthaptor	From genital atrium to opisthaptor	Between genital atrium and opisthaptor	Not stated	From genital atrium to opisthaptor

Table II-4 Comparisson of the species of *Pseudotagia* Yamaguti 1963 (continuation)

Author	<i>Pseudotagia cupida</i> (Hargis 1956) Yamaguti 1963	<i>Pseudotagia clathratus</i> Crane1972	Luque et al. 1993	<i>Pseudotagia rubri</i> Kohn et al. (1984)	Kohn et al. (1992)	<i>Pseudotagia n. sp.</i>
<b>Genital corona</b> (µm)	25 in diameter (20-28)	40 in diameter (20-50)	36 in diameter (31-38)	Not stated	36 long by 24 wide	43.4 of diameter (40-52.5)
<b>Number of genital spines</b>	6-7	6 fluted spines	6 (8 in one specimen)	6	6	7-8 (in almost the same proportion)
<b>Testes</b>	7-13	15-25	10-13	11-13 round	12	10-12
<b>Vagina</b>	Vaginae opens dorsolaterally anterior to ovary	Vaginae opens dorsolaterally, anterior to ovary	Vaginae with laterodorsal pores	Not stated	Not stated	vaginal pores laterodorsal (observed in just one individual and not clearly)
<b>Size of clamps</b>	Anterior clamps significantly larger, posterior clamps smaller	According to the drawing, appears to have the posterior pair of clamps are larger and the anterior pair the smallest	Two anterior pairs larger and equal size, third pair smaller and fourth pair smallest	Not stated, but the drawing indicated the two first pairs are of the same size, and the third pair is smaller and the last is smallest	The three first pairs are the same size and the last pair is smaller	The middle pairs of clamps are the larger, outer pairs (first and last pair) of similar size, with the first pair slightly larger than the last pair.
<b>Clamp edges</b>	Serrated	not serrated, highly muscular	Serrated	Serrated	Not stated	Not serrated
<b>Lappet</b>	One pair, but said not clearly seen	Two pairs	Two pair of hooks	Two pair of hooks	Not observed	Two pairs hook-like and side pair a domus
<b>Eggs</b>	One egg in the uterus badly distorted, <b>may have</b> short, stout filaments.	Not stated	Fusiform, with one filament at each pole 168 long by 78 wide (n=3), collapsed	Very large, with two short, stout polar filaments 44 long by 75 wide and filaments 120 long	190 long by 60 wide, with two filaments, the posterior filament is 180 long	One round and one slender pole, measures without filament 175 long by 68.3 wide. One long filament at slender pole

### Remarks

The species agrees with the characters of the genus *Pseudotagia*. This genus is composed of three species: *P. cupida* described by Hargis (1956) from the haemulid *Orthopristis chrysoptera* of the Gulf of Mexico, *P. clathratus* described by Crane (1972) from the serranid *Paralabrax clathratus* of the Pacific Ocean (California) and *P. rubri* described by Luque et al. (1993) from the haemulid *Orthopristis ruber* of the South Atlantic (Brazil). Kohn et al. (1984 and 1992) reported a pseudotagid monogenean from the haemulid *Haemulon sciurus* and the serranid *Diplectrum* sp. respectively and identified them as *P. cupida*. Luque et al. (1993) synonymized them as *P. rubri* based in the analysis of the information presented by Kohn et al (1984 and 1992). Table I-4 compares the differences or similarities between the four species of genus *Pseudotagia*.

In order to discuss the characters that distinguish the present specimens, we must first detail the basis for the differences in the three previously described species of *Pseudotagia* Yamaguti, 1963, these are *P. cupida* (Hargis, 1956), *P. clathratus* Crane, 1972 and *P. rubri* Luque et al. 1993.

**First:** Crane (1972) erected *Pseudotagia clathratus* based on the following differences with *P. cupida*: the smaller number of genital spines (which he refers to as atrial spines), the greater number of testes and the greater number of hooks in the lappet. Crane's species has 6 spines, while *P. cupida* has 6-7 spines. Hargis observed one pair of hooks in the lappet in *P. cupida*, but mentioned that they were not seen clearly. Among these characters, only the number of testes is significantly different, however, the sclerite composition of the clamps is very distinctive. The examination of photographs and drawings of the destroyed holotype material of *P. clathratus* allows us to observe, but not in detail some differences with *P. cupida*.

**Second:** Luque et al. (1993) erected *P. rubri* and differentiated it from *P. cupida* by the position of the vaginal pores and by the distributional pattern of the vitellaria. Hargis (1956) described the position of the vaginal pores at one-fourth of the body length from the anterior end and his drawing showed them at the same distance between the genital corona and the ovary, while in *P. rubri* they are at the level of the genital corona. The vitellaria in *P. cupida* occur from the level of the ovary to the opisthaptor, while in *P. rubri* this is from the the genital atrium to the opisthaptor.

The type material of *P. cupida* was lost and the vaginal pores in the heavily stained paratype of *P. rubri* were impossible to see. Considering that Luque et al. (1993) also did not have access to the type material of *P. cupida* and the imprecise description by Hargis (1956) of the clamp sizes, the number of hooks in the lappet, and the posterior limits of the intestinal caecae, these two species may be the same (see Table I-4).

### **Species level characters**

The new species described in this study differs from both species by the detail of the morphology of the lateral sclerites of the clamps and the relative sizes of the clamps. The lateral sclerites of the clamps of the new species are not serrated, as occurs in *P. cupida* and *P. rubri*. The relative size of the clamp pairs are different with the new species having the outer pairs (the first and the last pair) smaller than the central pairs, while *P. cupida* has the “anterior clamps significantly larger and the posterior clamps smaller and *P. rubri* has the first three pairs the same size and the last pair smaller.

The new species most closely resembles *P. rubri*, but differs, in addition to the characters mentioned above, by: 1. the intestinal caecae rejoin at the posterior of the body proper and extend into the opisthaptor as a single branch, while in *P. rubri* it is not confluent; and 2. the eggs observed in the new species has



one round pole without a filament and the other pole is slender with one long filament, while in *P. rubri* the eggs were described as fusiform.

The number of spines of the genital corona is insufficient to establish a distinction with the other species since the number of spines varied in each: in *P. cupida* Hargis (1956) observed 6-7 spines, in *P. rubri* Luque et al (1993) observed 6 spines in most of the worms and 8 in one specimen. In the new species 7-8 spines were observed.

### Conclusions

This study represents the first survey of macrovalvitrematid monogeneans for Puerto Rico (west and north coast) and the Caribbean. A total of 392 parasites distributed among Platyhelminthes (Digenea, Monogenea and Cestoda), Hirudinea (leeches), Nematoda, Copepoda and Isopoda were collected from 143 fishes of 12 genera and 18 species belonging to the families Sciaenidae, Haemulidae and Sparidae. The collection includes 352 monogeneans of which the 59% (207) corresponds to heteronchoinean monogeneans. The macrovalvitrematids were the most abundant 54% (189) of heteronchoinean monogenea collected and also the most abundant parasite taxa of the total of parasites collected with the 48% or 189 specimens.

This study establishes new records of the family Macrovalvitrematidae for Puerto Rico and the Caribbean. These include two new locality records for *Macrovalvitrema argentinensis* n. comb. and *Pterinotrematoides avaginata* n. comb., which previously have been reported from the same host (*Micropogonias furnieri*) from Mar del Plata, Argentina (south Atlantic); one new genus and species and one new species of the genus *Pseudotagia*. Also the creation of new combinations for the parasites mentioned above, establish the new locality record for the genera *Macrovalvitrema* and *Pterinotrematoides*.

The three families studied Sciaenidae, Haemulidae and Sparidae were infected by macrovalvitrematid monogenean, but only one species occurred in each family. [the sciaenid *Micropogonias furnieri* (Desmarest, 1823), the haemulid *Pomadasys corvinaeformis* (Steindachner, 1868) and the sparid *Archosargus rhomboidalis* (Linnaeus, 1758)]. *M. furnieri* hosted two macrovalvitrematid genera and species, *Macrovalvitrema argentinensis* n. comb and *P. avaginata* n. comb. In the haemulid *Pomadasys corvinaeformis* a new species of the genus *Pseudotagia* was found. This represents the first report of *Pseudotagia* in the Caribbean and also establishes the first record of macrovalvitrematid in haemulid of the genus *Pomadasys*. The genus *Pseudotagia* has been reported previously from the haemulids of the genera *Orthopristis* and *Haemulon*. A new and distinctive genus in family Macrovalvitrematidae was found in *A. rhomboidalis*. This also represents the first locality record of macrovalvitrematid for Puerto Rico and the Caribbean,.

None of the sciaenids *Bairdiella ronchus*, *Cynoscion jamaicensis*, *Larimus breviceps*, *Menticirrhus americanus*, *Umbrina broussoneti*, *U. coroides* and *Stellifer colonensis* were parasitized by macrovalvitrematids. The genus *Bairdiella*, specifically *B. chrysoura* has previously been recorded as a macrovalvitrematid host, but in this study only had polyonchoinean monogeneans of the genus *Rhamnocercus* and diclidophorid monogeneans of the genus *Pedocotyle*. *Menticirrhus americanus* and both species of *Umbrina* were hosts of polyonchoinean monogenea, while *C. jamaicensis*, *L. breviceps* and *S. colonensis* were free of gill parasites.

*Pomadasys corvinaeformis* was the only haemulid parasitized by macrovalvitrematids. The fishes of the genus *Haemulon* examined in this study only hosted polyonchoinean monogeneans and *Conodon nobilis* had no monogeneans. The few individuals of the genus *Calamus* examined only had polyonchoinean monogeneans.

The four macrovalvitrematid monogeneans had a prevalence greater than 50%, with the new species of *Pseudotagia* having the higher intensity (MI=3.77) and prevalence of 72%. The new genus and species found in *A. rhomboidalis* had a lower prevalence (54%).

The macrovalvitrematids studied have in common an unarmed genital atrium and copulatory organ armed with a genital corona or genital spines, wide anterior end and opisthaptor composed by eight pedunculated clamps arranged in pairs. The species of *Macrovalvitrema argentinensis* and *Pterinotrematoides avaginata* did not show any vaginal pores. The “genus n. and sp. n.” are distinguished by having a very large oral disc, square oral suckers, absence of pharynx, genital corona with plate-like armature and smaller and slender clamps. The new species of *Pseudotagia* recorded from *P. corvinaeformis* was distinguished by not having serrated lateral sclerites of their clamps as occurred in the other two species of *Pseudotagia* reported from haemulid fishes.

This study also includes the following observations not described before in any macrovalvitrematid monogeneans:

- › The clamps of *Macrovalvitrema*, *Pterinotrematoides* and the “genus n. and sp. n.” have an asymmetrical plate along the ventral valve with a thin fissure in one side.
- › This is modified in the “genus n. sp. n.” because the reduction of the width in the proximal part of their clamps. The pairs of clamps in *Macrovalvitrema* and *Pterinotrematoides* are arranged as mirror images of the other. This is also seen in the small pair of clamps of the “gen. n. sp. n.”
- › The basal sclerite of the large clamps can cover the proximal part of the lateral sclerites of the ventral valve or can be fused laterally with it.

### Literature Cited

- Alves, D.R. and J.L. Luque. 2000. Metazoarios parásitos de *Micropogonias furnieri* (Osteichthyes: Sciaenidae) do litoral do Estado do Rio de Janeiro, Brasil. *Parasitologia al Dia* 24: 40-45.
- Alves, D. R. and J. L. Luque. 2001. Community ecology of the metazoan parasites of white croaker, *Micropogonias furnieri* (Osteichthyes: Sciaenidae), from the Coastal zone of the State of Rio de Janeiro, Brazil. *Memorias del Instituto Oswaldo Cruz* 96: 145-153.
- Boeger, W. A. and D. C. Kritsky. 1993. Phylogeny and a revised classification of the Monogenoidea Bychowsky, 1937 (Platyhelminthes). *Systematic Parasitology* 26: 1-32.
- Boeger, W. A. and d. C. Kritsky. 2001. Phylogenetic Relationships of the Monogenoidea. *In* Interrelationships of the Platyhelminthes, D. T. J. Littlewood and R. A. Bray (eds.). Taylor and Francis, London, U. K., p. 92-102.
- Bravo-Hollis, M. 1982. Helmintos de peces del pacifico mexicano XXXIX. Dos subfamilias nuevas de Monogéneos de la familia Macrovalvitrematidae Yamaguti, 1963. *Anales del Instituto de Biología Universidad Nacional Autónoma de México* 52: 27-38.
- Bychowsky, B. E. 1957. Monogenetic trematodes their systematic and phylogeny. English translation of Russian monograph, Hargis, W. J. American Institute of Biological Sciences (1961). 627 pp.
- Bychowsky, B. E. and L. Nagibina. 1959. On the representative of monogenetic flukes from the South China sea. *Acta Zoologica. Sinica* 11: 211-231.
- Caballero y C. y Bravo-Hollis, 1955. Trematodos de peces marinos de aguas mexicanas del Océano Pácifico. VIII. Descripción de tres nuevos generos de trematodos monogéneos. *Anales del Instituto de Biología* 16: 89-115.
- Centeno, L., A. K. Bashirullah, M. E. Alvarez and R. Alvarez. 2002. Análisis comparativo de las comunidades de parásitos metazoarios en dos especies de peces marinos del Golfo Cariaco, Venezuela. *Bioagro* 14: 135-144.
- Crane, J. W. 1972. Systematics and New Species of Marine Monogenea from California. *The Wasmann Journal of Biology* 30: 109-166.
- Dowgiallo, M. J. 1979. Variation of metazoan parasites of the French grunt, *Haemulon flavolineatum* (Desmarest) (Osteichthyes: Pomadasysidae), by habitat type and season with an analysis of competition among parasites.
- Fernandez, B. M. M., A. D. N. Arci, S. C. Cohen. 2009. New data on some species of

- Monogenea and Digenea parasites of marine fish from the coast of the State of Rio de Janeiro, Brazil. *Rev. Bras. Parasitol. Vet. Jaboticabal* 18: 13-18.
- Fuentes Zambrano, J. L., I. Sprock, Y. Mago y O. L. Chinchilla. 2009. Monogeneos parásitos de peces de la laguna Las Marites, Isla de Margarita, Venezuela. *Interciencia* 34: 507-513.
- Gusev, A. V. 1976. Monogenoidea of Freshwater Fishes. Principles of Systematics, Analysis of World Fauna and its Evolution. *Parazitologicheskii Sbornik* 28: 96-198 [In Russian]
- Hargis, W. J. Jr. 1956. Monogenetic Trematodes of Gulf of Mexico Fishes. Part VIII. The Superfamily Diclidophoroidea Price, 1936. (Continued). *Helminthological Society* 23: 5-13.
- Kearn, G. C. 1998. Parasitism and the platyhelminths. 1<sup>st</sup> ed. Chapman & Hall. 544 pp.
- Kohn, A. and S. C. Cohen. 1998. South American Monogenea-list of species, host and geographical distribution. *International Journal for Parasitology* 28: 1517-1554.
- Kohn, A. and M. Pinto-Paiva. Fishes parasitized by Monogenea in South America. In: Salgado-Maldonado, G., A. N. García-Aldrete and V. M. Vidal-Martínez (Eds.). 2000. Metazoan parasites in the neotropics: a systematic and ecological perspective. Instituto de Biología, UNAM. Mexico. P. 25-50.
- Kohn, A., B. Abramson and B. Macedo. 1984. Studies on some monogenean parasites of *Haemulon sciurus* (Shaw, 1803) (Pomadasyidae). *Journal of Helminthology* 58: 213-218.
- Kohn, A. C. P. Santos and M. F. D. Baptista-Farias. 1992. New host records and localities of some Monogenea from Brazilian marine fishes with scanning electron microscopy of Bicotylophora trachinoti (MacCallum, 1921). *Mem Ist. Oswaldo Cruz* 87: 109-114.
- Kohn, A., C. Portes Santos & S. Chinickz Cohen. 1989. Monogenea parasites of *Micropogonias furnieri* (Desmarest, 1823) (Pisces, Sciaenidae) from the litoral of Rio de Janeiro State, Brazil. *Memorias del Instituto Oswaldo Cruz, Rio de Janeiro* 84, Supple. IV: 291-295.
- Lebedev, B. I. 1979. Faunistic aspects of studies in the higher Monogenoidea of marine fishes. *Zool. Anz. Jena.* 202 (1/2) S: 99-104.
- Llewellyn, J. 1954. Observations on the food and the gut pigment of the Polyopisthocotylea (Trematoda: Monogenea). *Parasitology* 44: 428-437.
- Luque, J. L., J. F. R. Amato and R. M. Takemoto. 1993. A New Species of *Pseudotagia* Yamaguti, 1963 (Monogenea: Macrovalvitrematidae) Parasitic on *Orthopristis ruber* (Cuvier) (Osteichthyes: Haemulidae) from the Brazilian Coast. *Rev. Bras. Parasitol. Vet. (Brazil. J. Vet. Parasitol.)* 2: 111-114
- Mamaev, Y. L. 1976. [The system and phylogeny of monogeneans of the family

- Diclidophoridae]. In: Fauna and systematic and phylogenetics of Monogenoidea. Trudy biologo-Pochvennogo Instituta, Novaya Seriya 35: 57-80. (In Russian).
- Mamaev, Y. L. and Lebedev, B. I. 1979. The system of higher monogeneans in the light of recent knowledge. Zoologica Scripta 8: 13-18.
- Payne, R. R. 1987. Two New Monogenea (Macrovalvitrematidae) From Eastern Pacific Ocean Fishes.
- Pearse, A. S. 1949. Observations on Flatworms and Nemerteans Collected at Beaufort, N. C. Proceedings of the United States National Museum 100: 25-38.
- Poulin R. 2002. The evolution of monogenean diversity. International Journal of parasitology. 32:245-254.
- Roberts, L. S. and J. Janovy Jr. 2009. Gerald D. Schmidh & Larry S. Roberts' foundations of parasitology. 8<sup>th</sup> ed. McGrawHill Higher Education. 701 pp.
- Santos, C. P. and C. A. A. Carbonel H. In. Salgado-Maldonado, G., A. N. García-Aldrete and V. M. Vidal-Martínez (Eds.). 2000. Metazoan parasites in the neotropics: a systematic and ecological perspective. Instituto de Biología, UNAM. Mexico. P. 61-75.
- Spencer Jones, M. E. and D. I. Gibson. 1990. A list of old and recently erected monogenean genus-group names not included in Yamaguti's *Systema helminthum*. Systematic Parasitology 16: 213-226.
- Suriano, D. M. 1966. Estudio de la fauna parasitaria de *Micropogon opercularis*. En relación con problemas zoogeográficos del Atlántico sur.
- Suriano, D. M. 1975. Sistemática, biología y microecología de tres Monogenea, Polyopisthocotylea parásitos de las branquias de *Micropogonias opercularis* (Quoy y Gaimard) y *Umbrina canosai* Berg (Pisces, Sciaenidae) del Océano Atlántico Sudoccidental. Physis. Sección A; Los Océanos y sus organismos 34: 147-163.
- Tantaleán, M. V., R. R. Martínez y A. H. Escalante. 1985. Monogéneos de las costas del Perú. II. Cambio de nombre por homonimia y nuevos registros. Revista de la Facultad de Ciencias Veterinarias 32: 91-95.
- Yamaguti, S. 1963. Systema Helminthum Volume IV. Monogenea and Aspidocotylea. Interscience, London, 699 pp.

Plate 1. *Macrovalvitrema argentinensis* (Suriano, 1975) (New material) from the Whitemouth croaker *Micropogonias furnieri* Desmarest 1823: **a.** ventral view of complete body; **b.** side view of complete body; **c.** anterior end of the body showing both rims of the oral disc (OD) covered by several glands, the protuberance in the dorsal rim (dr) and the inward folds in the ventral rim (vr), the oral suckers (OS) with their muscular base and the pharynx (Ph); **d.** anterior end of the body showing the mouth (M) opens, the oral suckers (OS) and the oral disc (OD), also is observed the genital atrium (GA) and the intestinal bifurcation (IB); **e.** the oral disc open allowing the oral suckers adhere to the substrate; **f.** the egestion of the food (f) through the mouth.

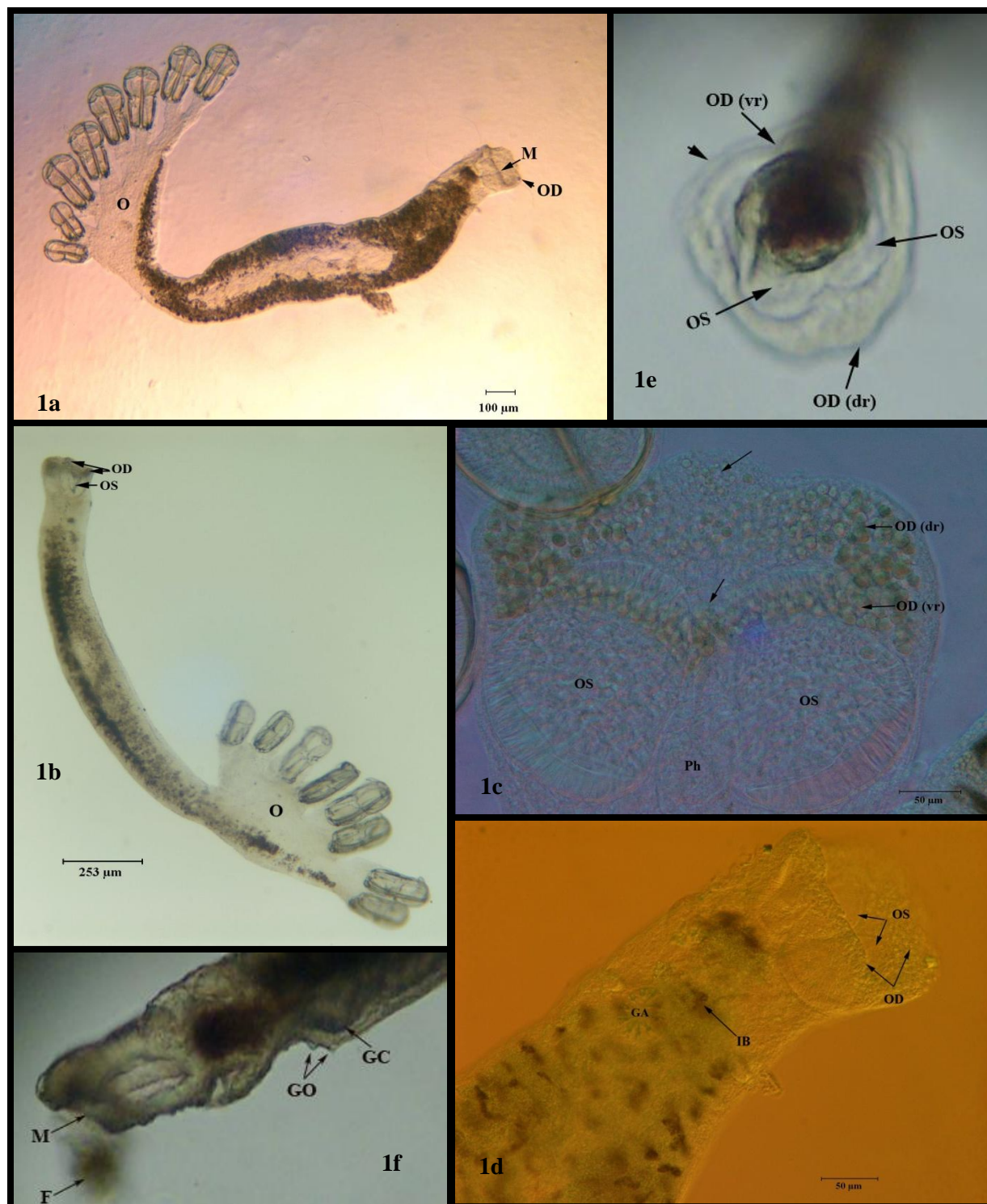
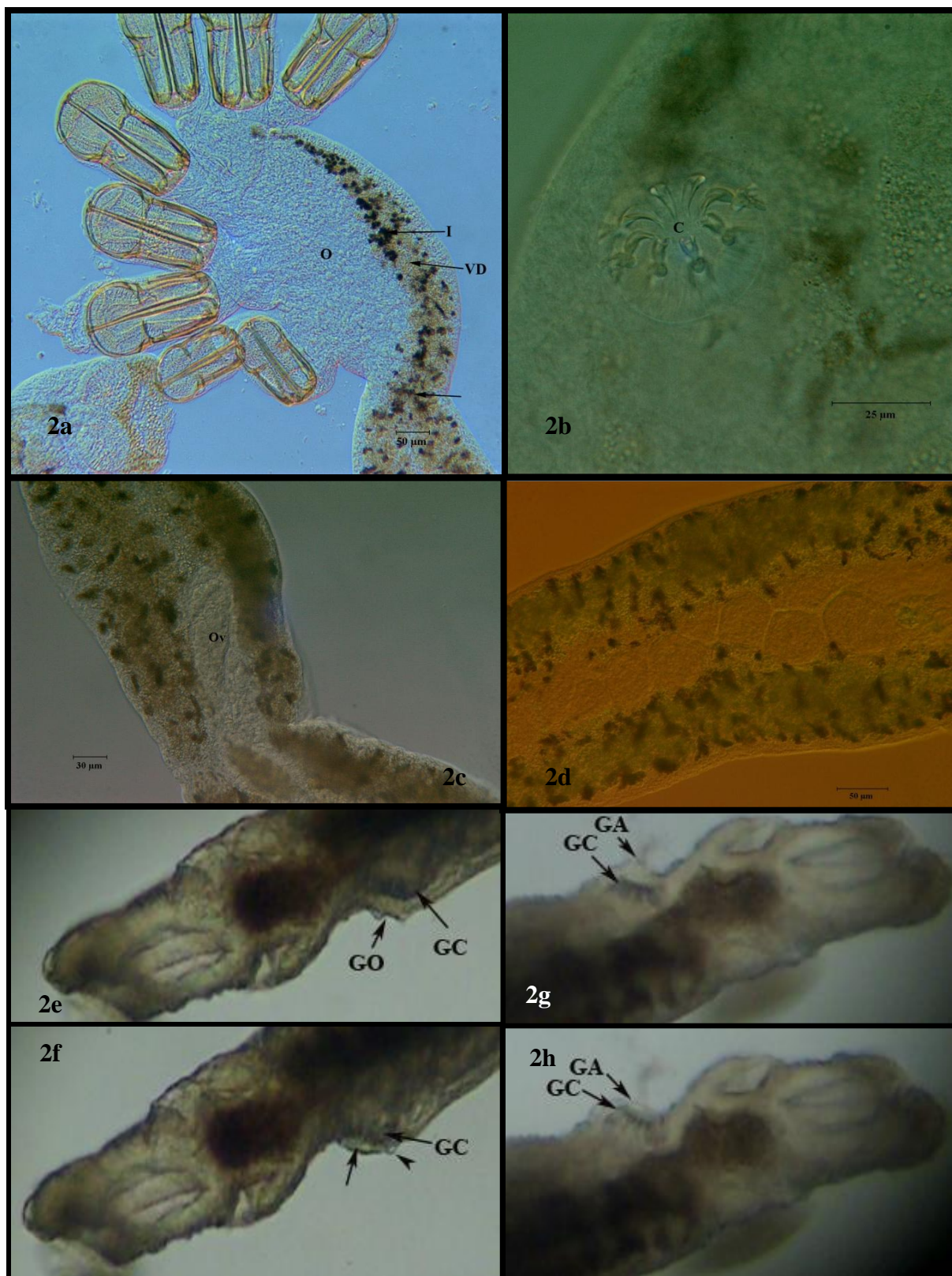


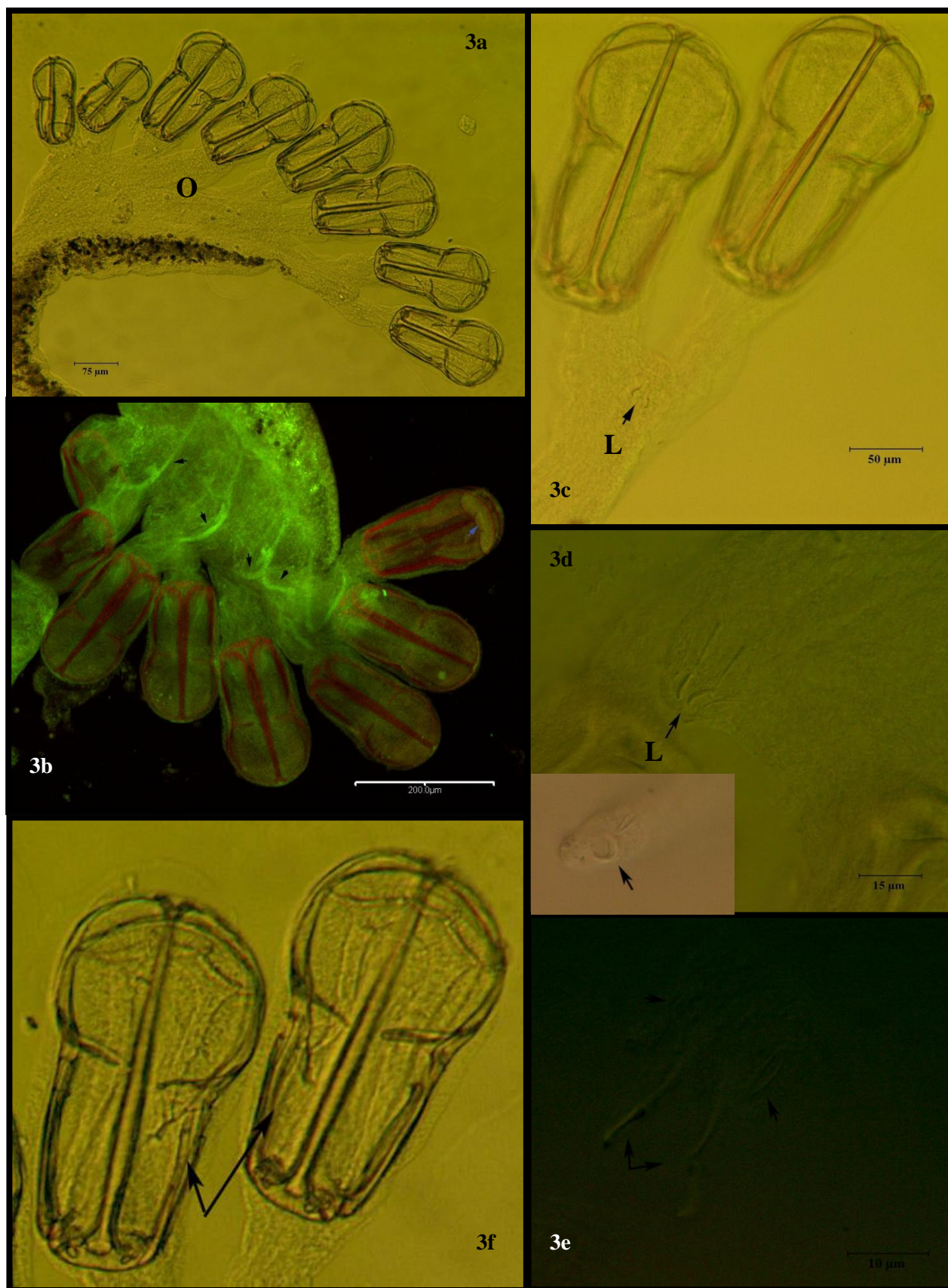


Plate 2. *Macrovalvitrema argentinensis* (Suriano, 1975) New material from the Whitemouth croaker *M. furnieri*: **a.** joining of the intestine (I) and vitellaria (VD) anterior to the opisthaptor entering into the opisthaptor (O) as one dorsal branch; **b.** Genital corona of male copulatory organ (GC)); **c.** testes; **d.** ovary (O); **e.** lips of the genital opening (GO) (when this is closed), also is visible the genital corona inside the body and behind the genital opening; **f.** genital opening open indicated by the lips in the side of the opening by the arrow (↖); **g** genital corona (GC) inside the body; **h.** spines of the genital corona oriented toward the genital opening.

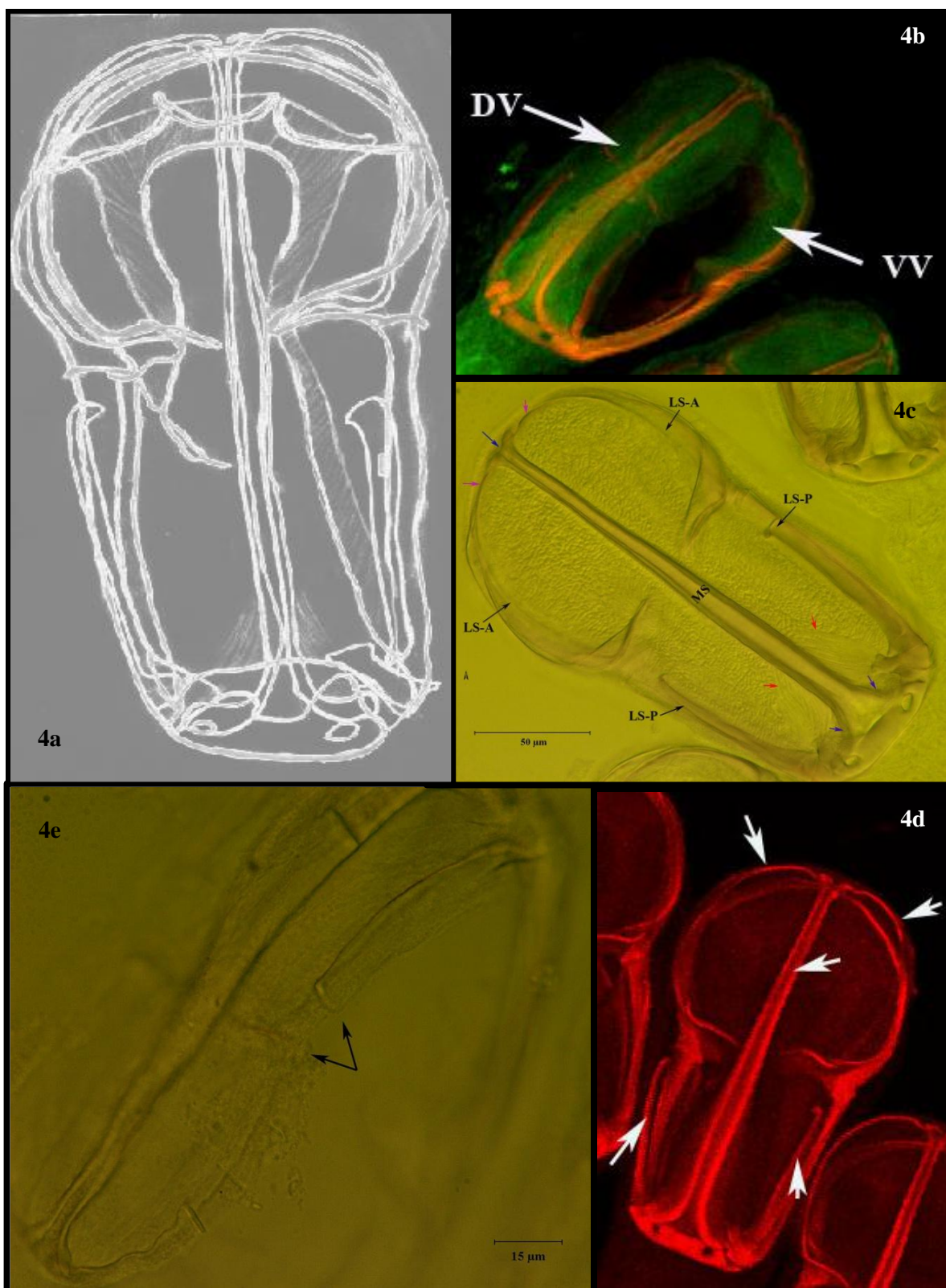










**Plate 3. *Macrovalvitrema argentinensis* (Suriano, 1975) a-b.** opisthaptor (O) ; **c-e.** posteriormost pair of clamps with the lappet (L) between their peduncles; lappet with two pair of hooks; **f.** mirror image arrangement of the pairs of clamps.



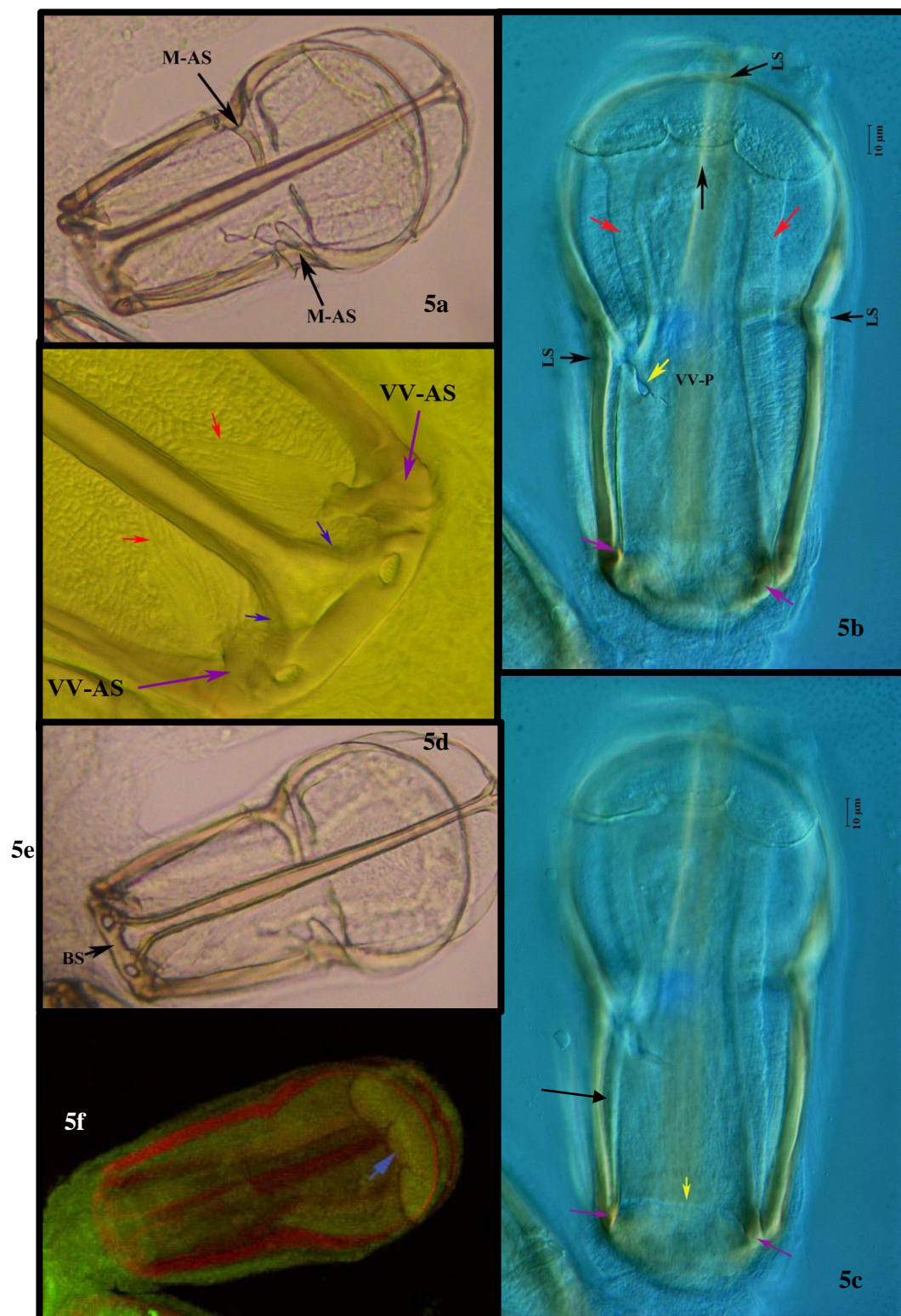


**Plate 4. *Macrovalvitrema argentinensis* (Suriano, 1975)** **a.** drawing of the clamp; **b.** dorsal and ventral valves (DV) and (VV) respectively; **c.** sclerites of dorsal valve including the distal and proximal segment of the lateral sclerites (LS) and the median sclerite (MS); **d.** complete clamp with confocal microscopy; **e.** separation of lateral sclerite of dorsal valve at strangulation level



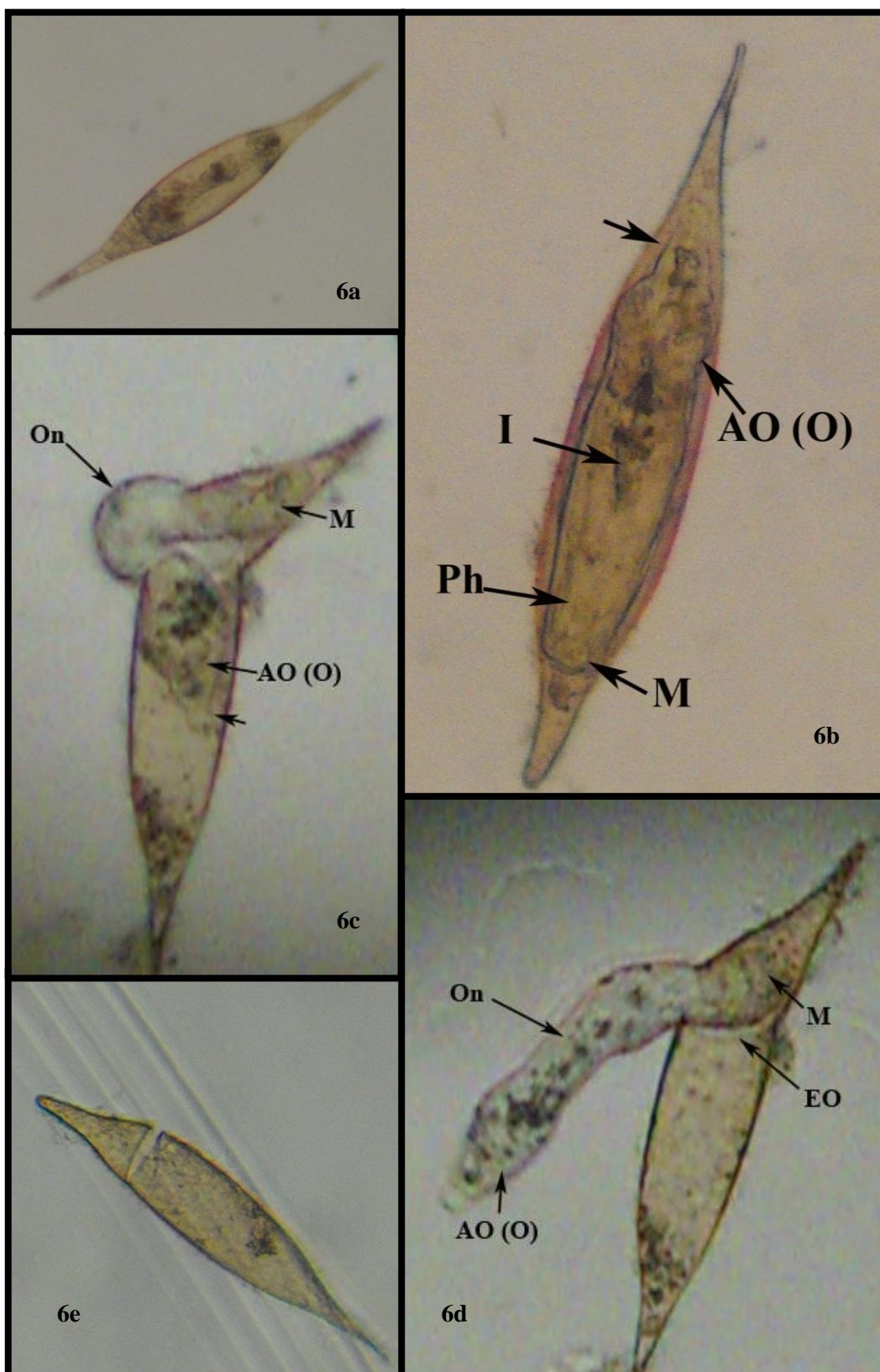
**Figure 5** *Macrovalvitrema argentinensis* (Suriano, 1975). **a.** accessory sclerites (AS) of the lateral sclerites of ventral valve; **b.** sclerites and other structures of the ventral valve (VV), including the lateral sclerites (LS) and the plate, which covers almost the complete extend of the valve, the red arrows (  ) indicates the muscular wall that surrounds this plate and the yellow arrow (  ) the suture or fissure in one of the side of the plate (this fissure is what attribute the mirrow image arrangement of the clamps). The purple arrow (  ) indicates the articulation of the proximal portion of the plate around the lateral sclerites of the ventral valve, and the black arrow (  ) point the scalloped muscular pad in the distal portion of the ventral valve; **c.** ventral valve, the black arrow (  ) is pointing the lateral sclerites, the yellow arrow (  ) point the end of the plate, which medially does not articulate with the basal sclerite and the purple arrow (  ) is pointing the articulation of the plate to the lateral sclerites; **d.** basal sclerite (BS); **f.** picture of a clamp using confocal microscopy, the blue arrow (  ) is pointing the scalloped muscular pad of the distal end of the ventral valve.



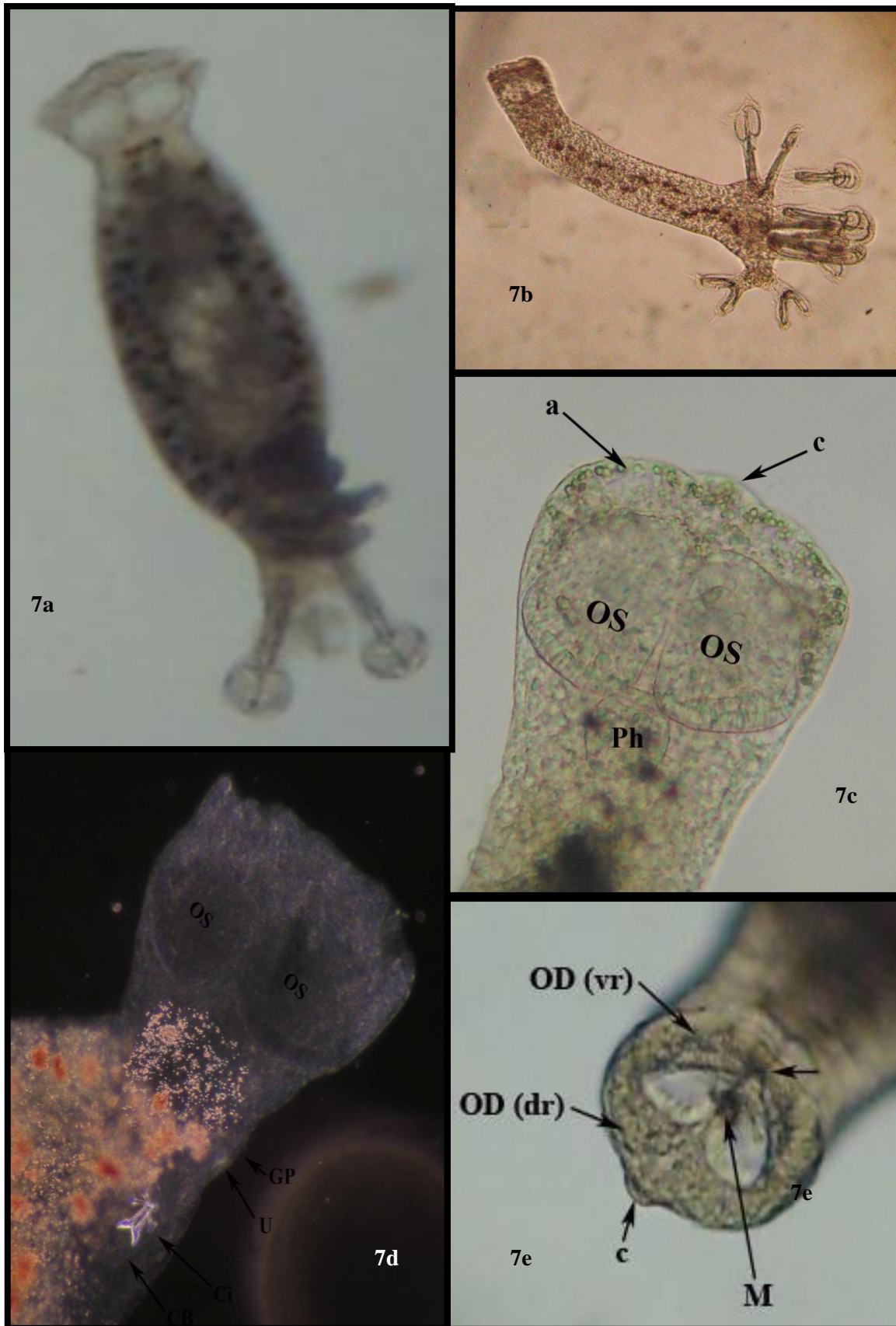




**Plate 6. *Macrovalvitrema argentinensis* (Suriano, 1975).** **a-b.** Different stages of larva development. In the figure **b** is pointed the attachment organ (AO) or the opisthaptor (O), intestine (I), pharynx (Ph) and the mouth (M) of the larva; **c-d.** hatching of the larva or oncomiracidium (On) through the egg operculum (EO). **E.** egg broken.

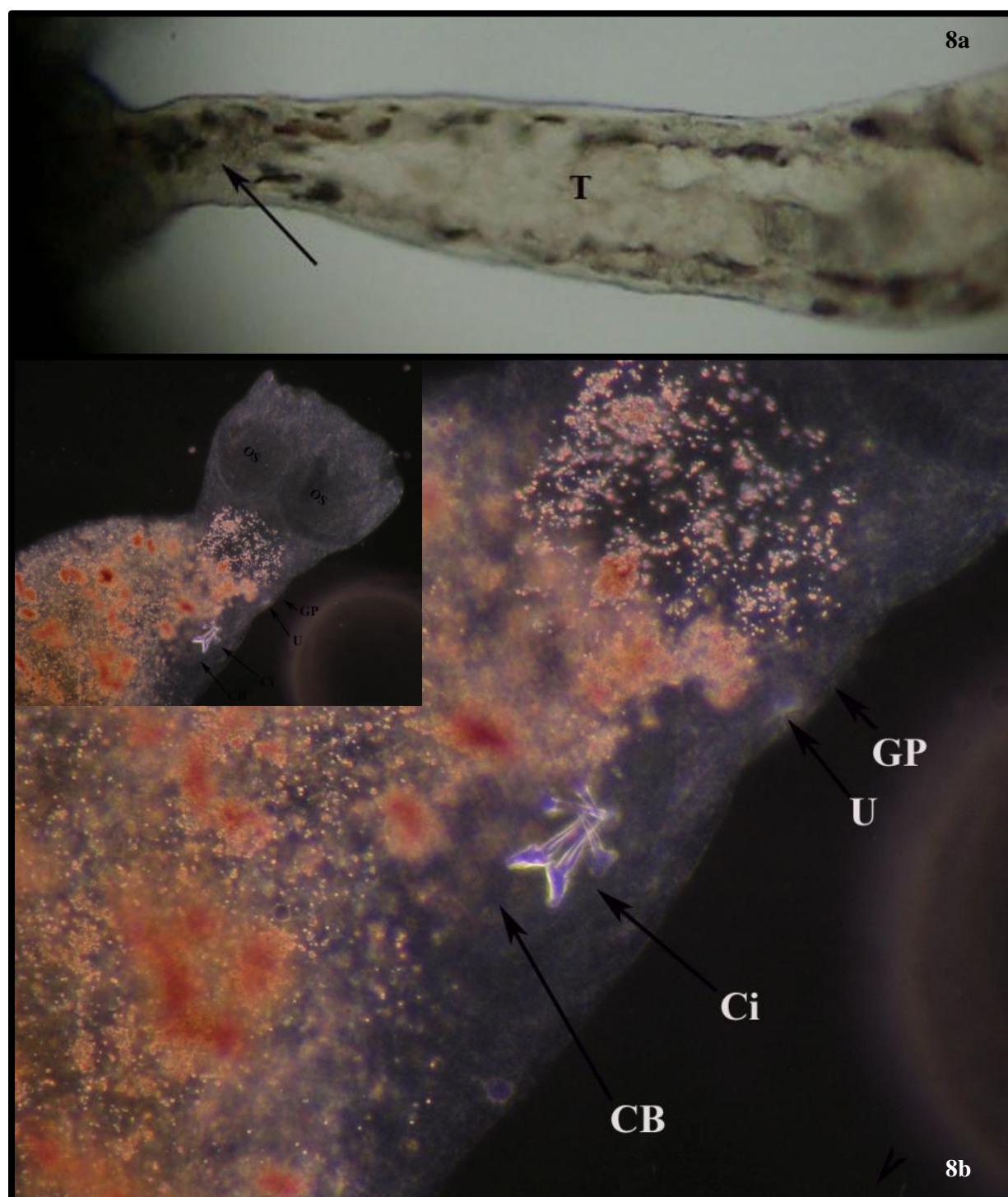


**Plate 7. *Neopteriotrematoides avaginata* Suriano, 1975).** New material from the whitemouth croaker *Micropogonias furnieri*. **a-b.** adult; **c-d.** anterior end, in the figure c is showing the oral suckers (OS), the pharynx (Ph), the letters a and c point the glands around the oral disc and the protuberance in the dorsal side of the oral disc respectively. **e.** anterior end from above, is showing both sides of the oral disc (OD), ventral rim (vr) and dorsal rim (dr), the mouth with a heart shaped.

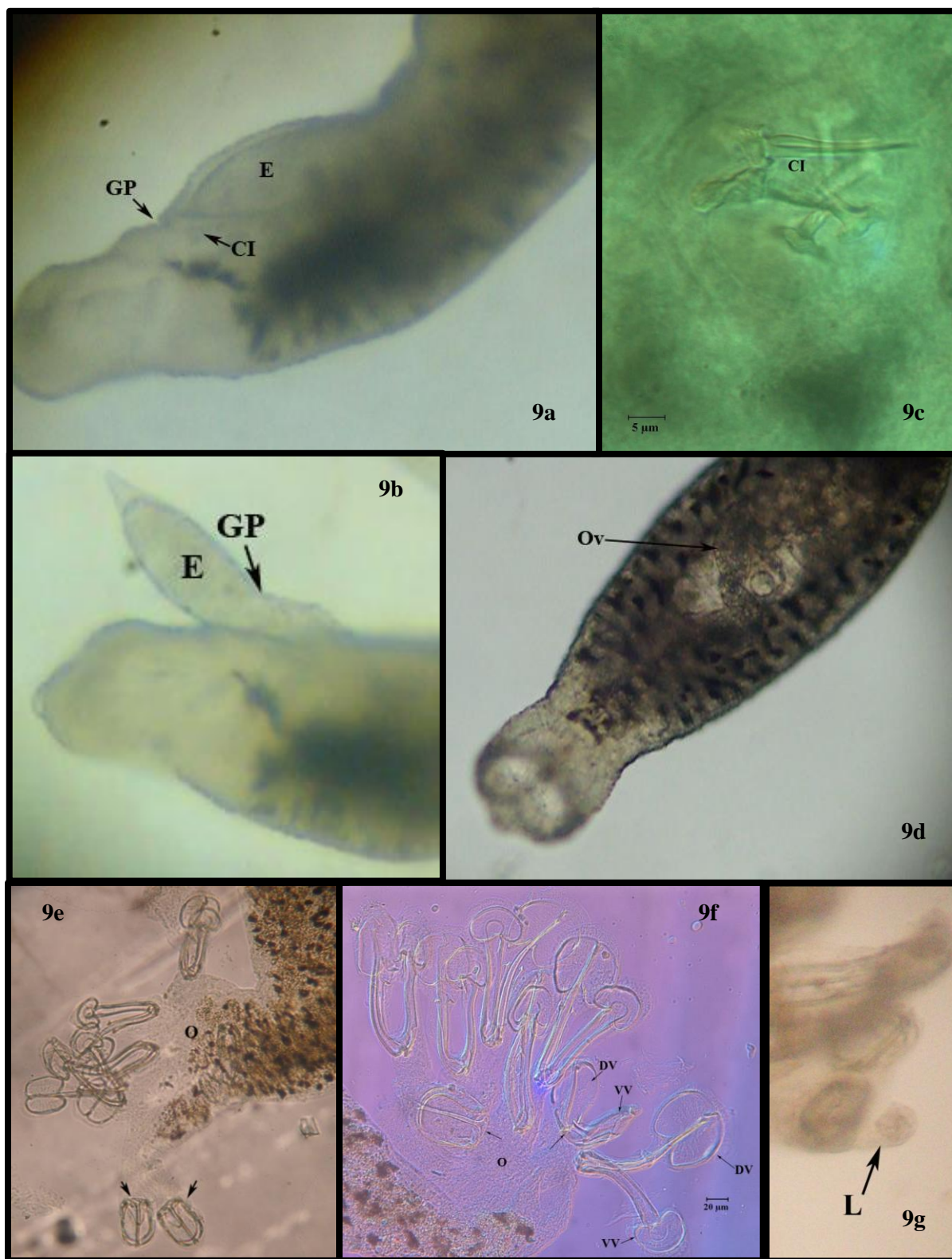


**Plate 8.** *Neopteriotrematoides avaginata* Suriano, 1975. Genitalia. **a.** testes (T) and the black arrow indicate the rejoin of the intestinal cecae; **b.** shows the spines of the copulatory organ or cirrus (Ci), their cirrus bulb (CB) and the end of the uterus (U) close to the genital pore (GP).



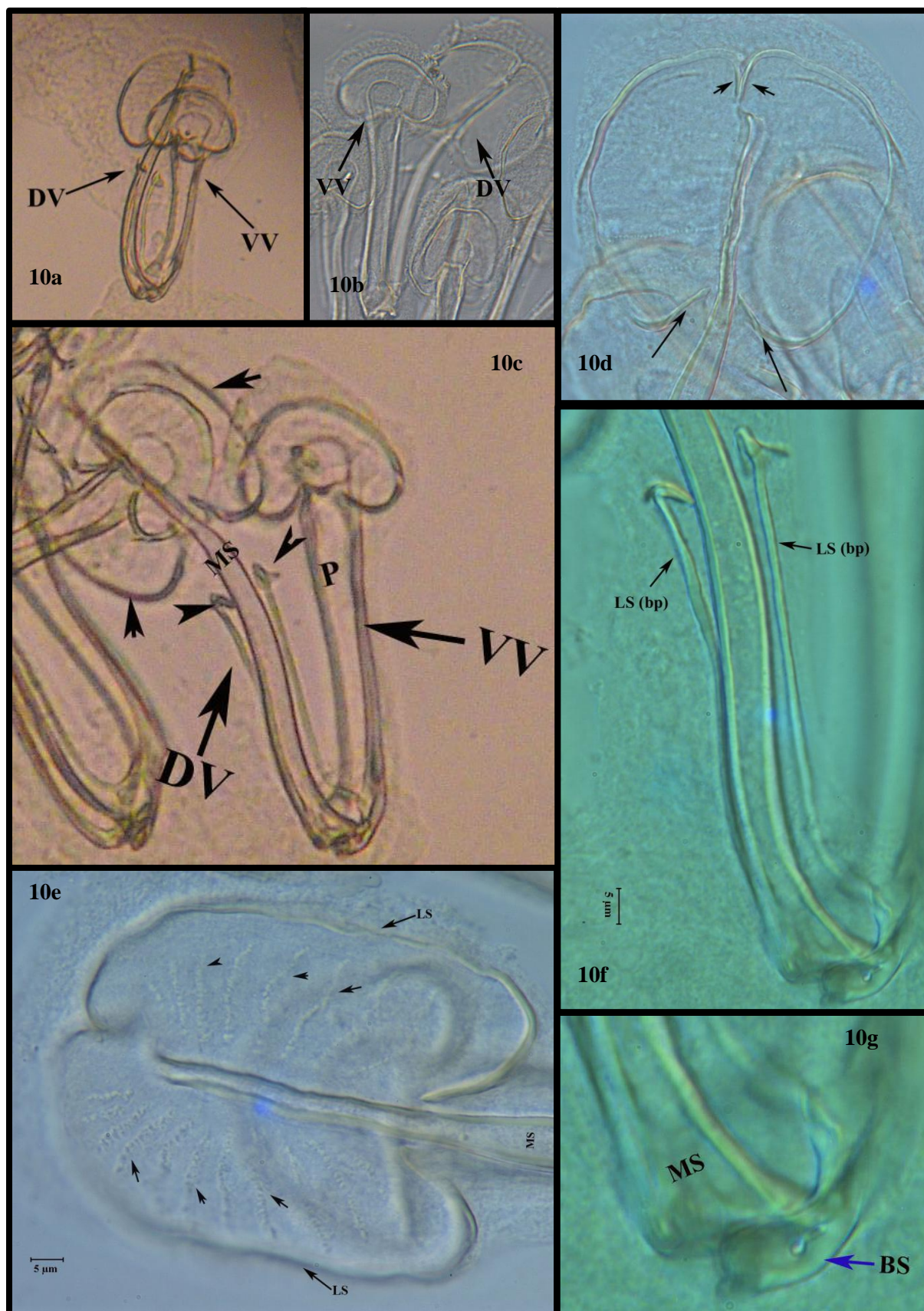


**Figure 9.** *Neopterinotrematoides avaginata* Suriano, 1975. **a-b.** is showing the ovoposition through the genital pore (GP); **c.** the spines of the male copulatory organ or cirrus (Ci) which are oriented from different positions; **d.** ovary (O); **e-f.** opisthaptor (O) showing some clamps opens exposing their ventral and dorsal valves (VV) and DV) respectively; **g.** lappet (L).



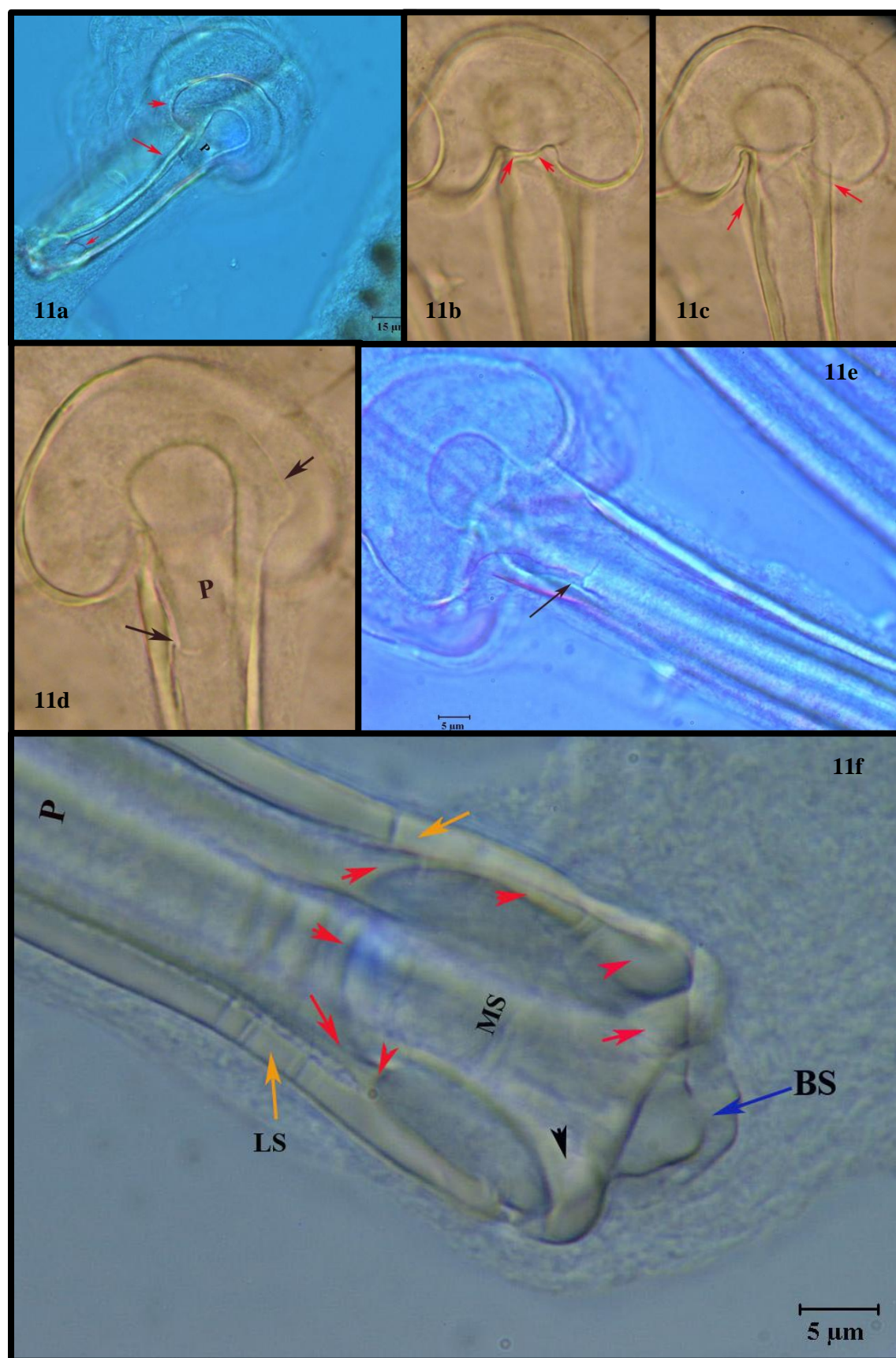


**Plate 10. *Neopteriotrematoides avaginata* Suriano, 1975.** Large clamps. **a-c.** dorsal and ventral valve (dv) and (vv) respectively, the picture c point all the sclerites that belongs to the dorsal valve, the median sclerite (MS) and the black arrows refers to the distal and proximal segments of the lateral sclerite of that valve; of the ventral valve is showing the plate (P); **d.** distal segment of dorsal valve, and is pointing the distal and median ends of the distal segment of the lateral sclerite; **e.** the distal segment of the lateral sclerite (LS) of the dorsal valve and the small arrows are pointing the rows of bars of this part of the dorsal valve; **f.** proximal segments of lateral sclerites of dorsal valve, which ends in a marked curved tips; **g.** articulation of the median sclerite (MS) of dorsal valve with the basal sclerite (BS).

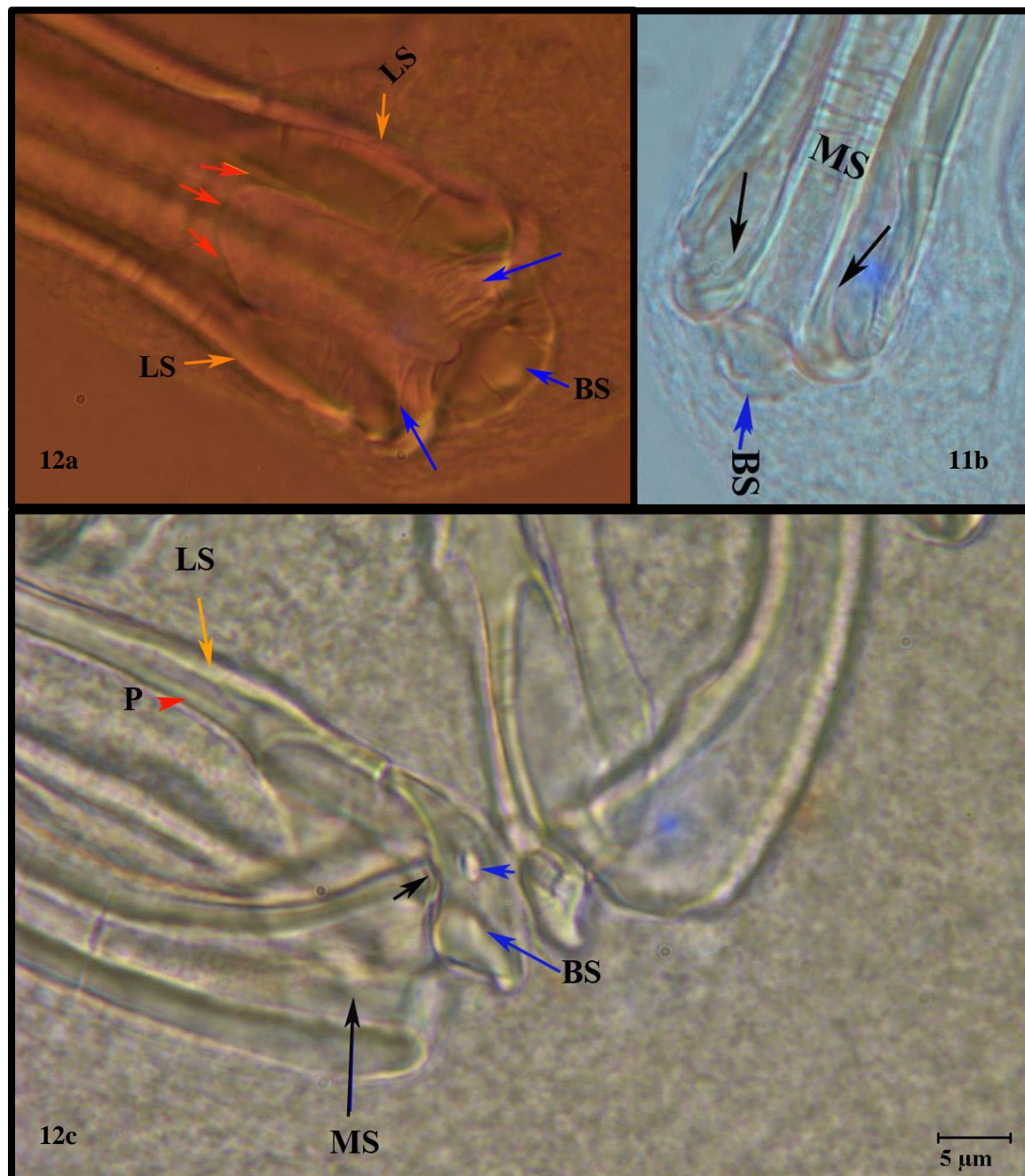


**Figure 11.** *Neopterinotrematoides avaginata* Suriano, 1975. Ventral valve of the large clamps. **a.** the wide and slender parts of the lateral sclerite; **b.** accessory sclerites at strangulation level; **c.** strangulation level of the ventral valve; **d-e.** plate (P) the anterior arrow indicates the muscular wall that is surrounding the plate in the distal portion of the valve and the posterior and the only for the picture e is pointing the suture or fissure in one of the side of the plate in the proximal or slender portion of that valve; **f.** basal area of the valve, inner or ventral view of the tip of basal sclerite (BS), the yellow arrows (→) point the lateral sclerite of ventral valve, the red arrows (→) point the end and the articulation of the plate (P) with the lateral sclerites of ventral valves and through the space medially between the end of the plate and the basal sclerite is visible the median sclerite (MS) of the dorsal valve, the black arrows (→) indicates the end of the median sclerite at the basal sclerite.



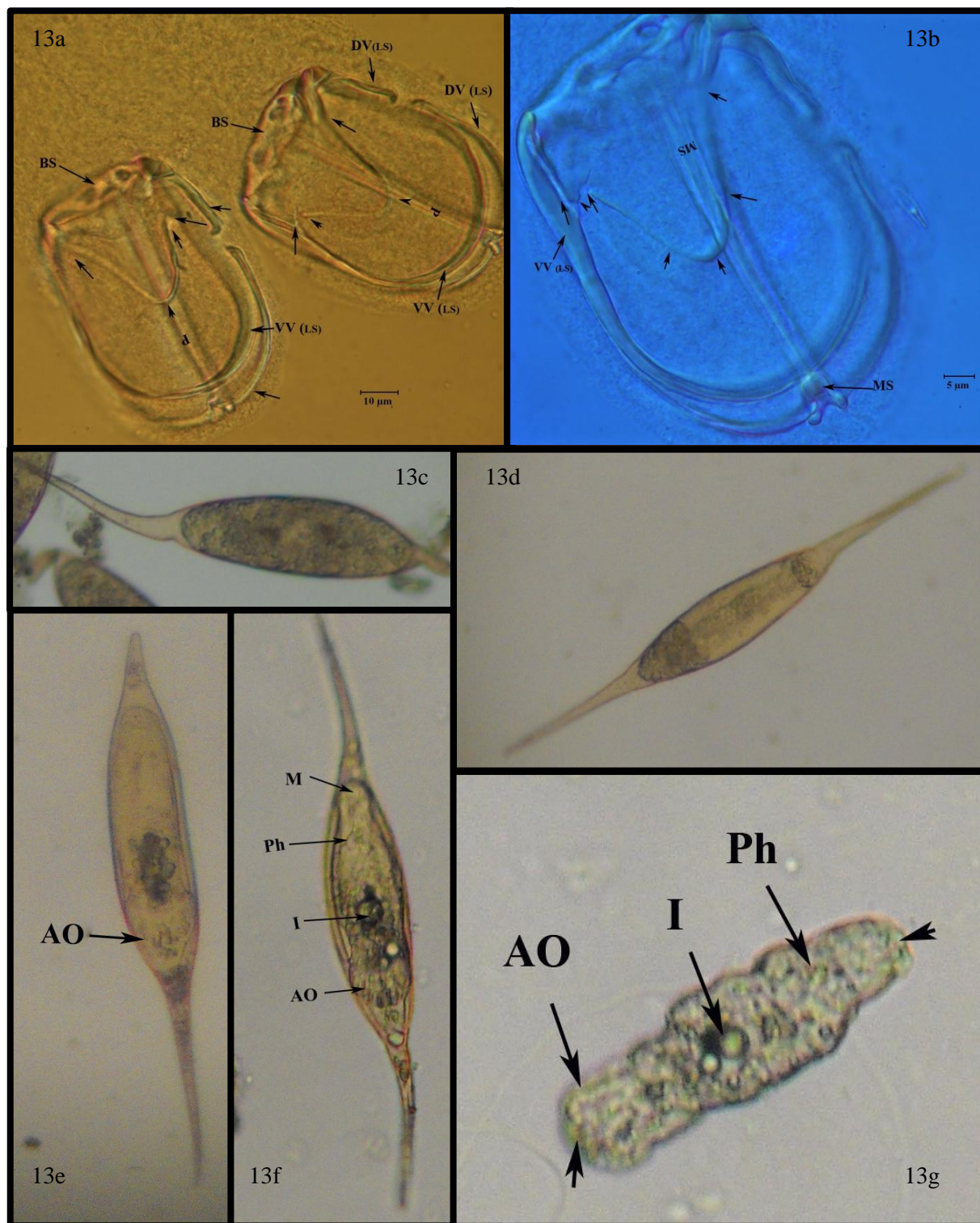


**Plate 12. *Neopteriotrematoides avaginata* Suriano, 1975.** Basal region of the large clamps. **a.** articulation of the basal sclerite (BS) on the lateral sclerites of the ventral valve, the yellow arrows are pointing the lateral sclerites, the blue arrows points how the basal sclerite covers the lateral sclerite of ventral valve and do not cover the median area of this side of the clamp and the red arrows are pointing the end of the plate. The edges of the basal sclerite and the plate ends create a space between them through what is visible the median sclerite of the dorsal valve; **b.** articulation of basal end of median sclerite with the basal sclerite; **c.** side view of the articulation of the median sclerite of dorsal valve and the basal sclerite.



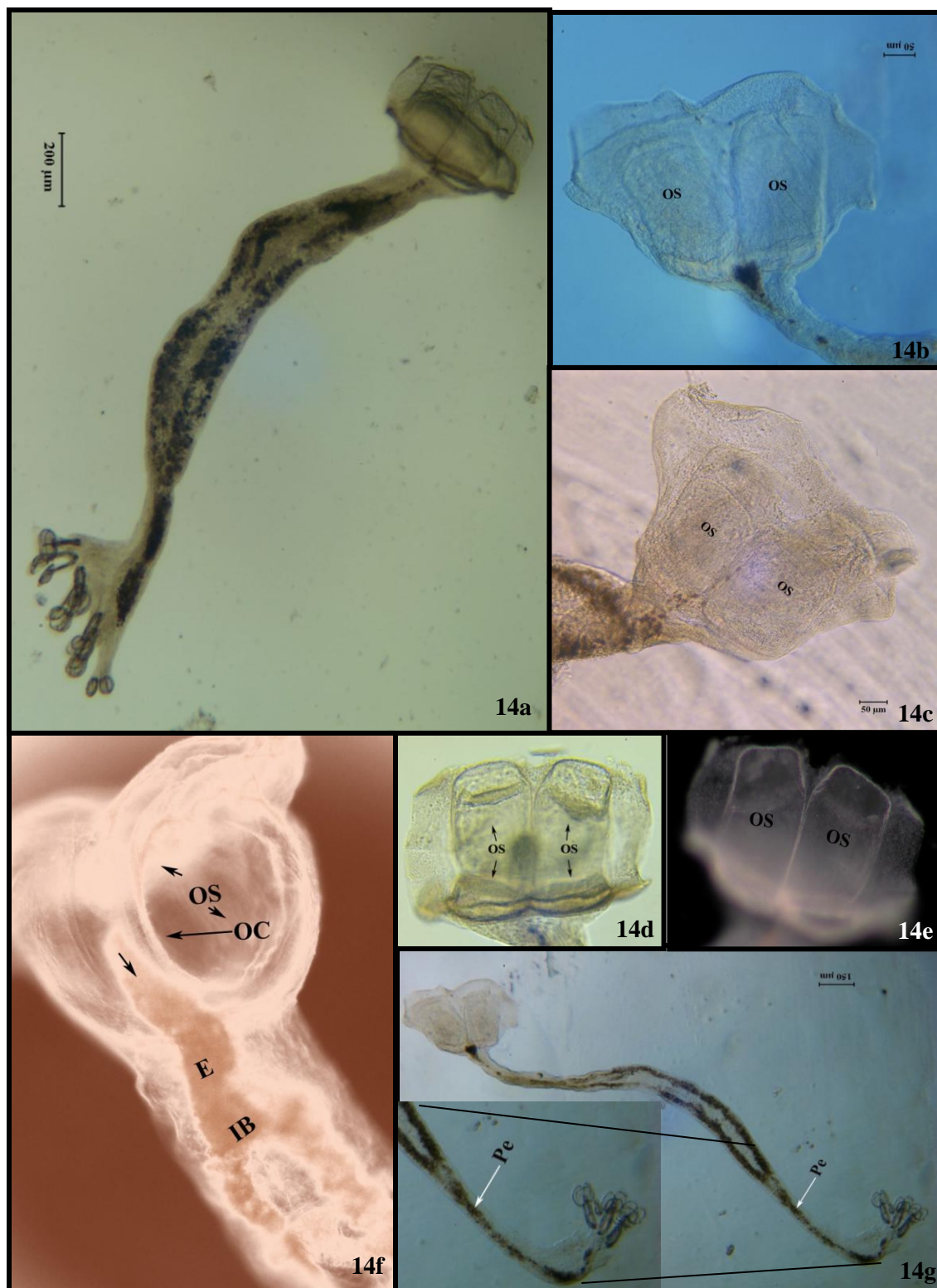
**Plate 13.** *Neopteriotrematoides avaginata* Suriano, 1975. **a-b.** small clamps. The abbreviations observed in the small clamps corresponds to the same discussed above in the large clamps. **c-f.** different stages of the larva or oncomiracidium and is pointed their mouth (M), their pharynx (Ph), intestine (I) and the attachment organ (AO); **g.** larva or oncomiracidium.



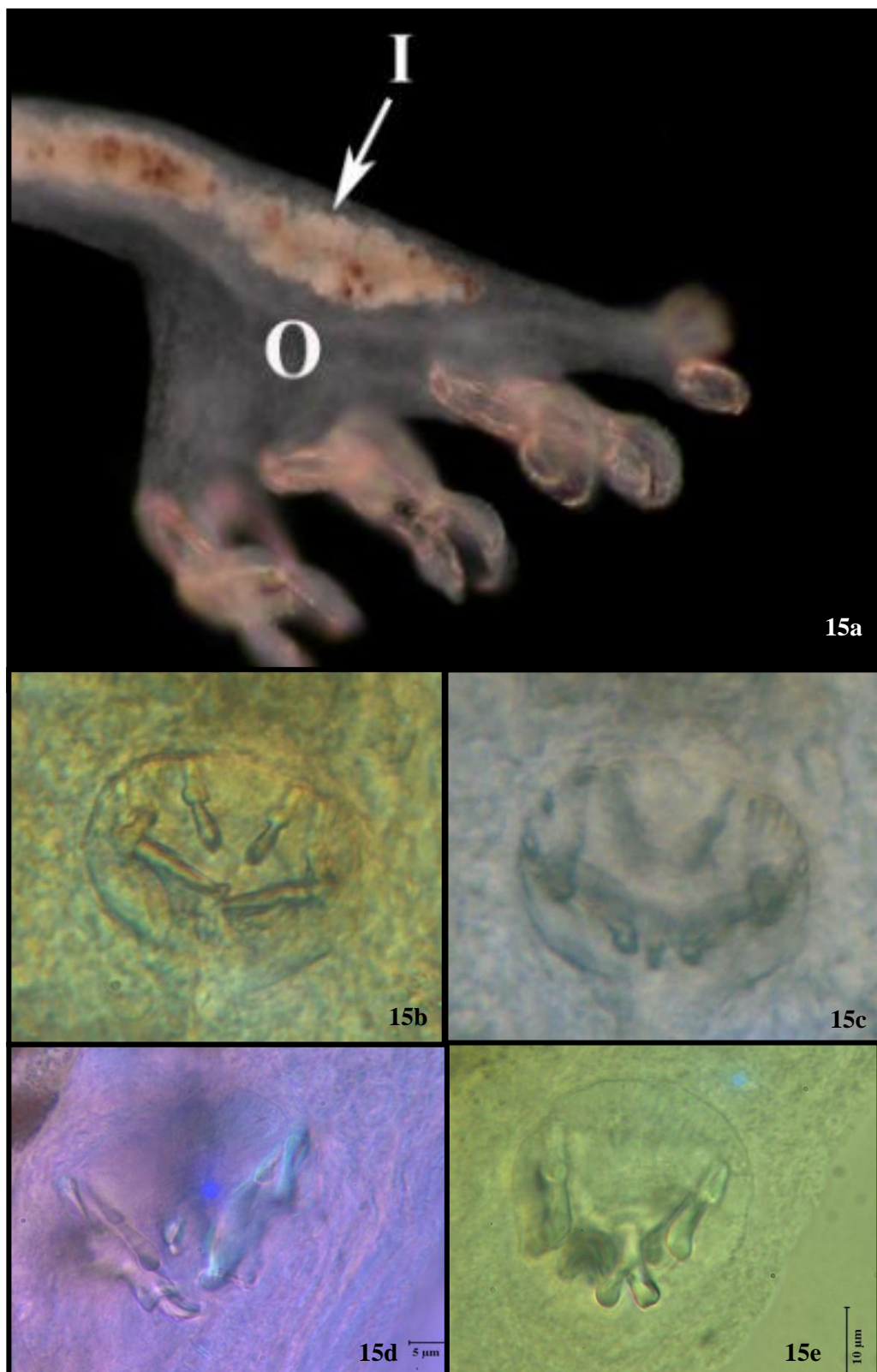




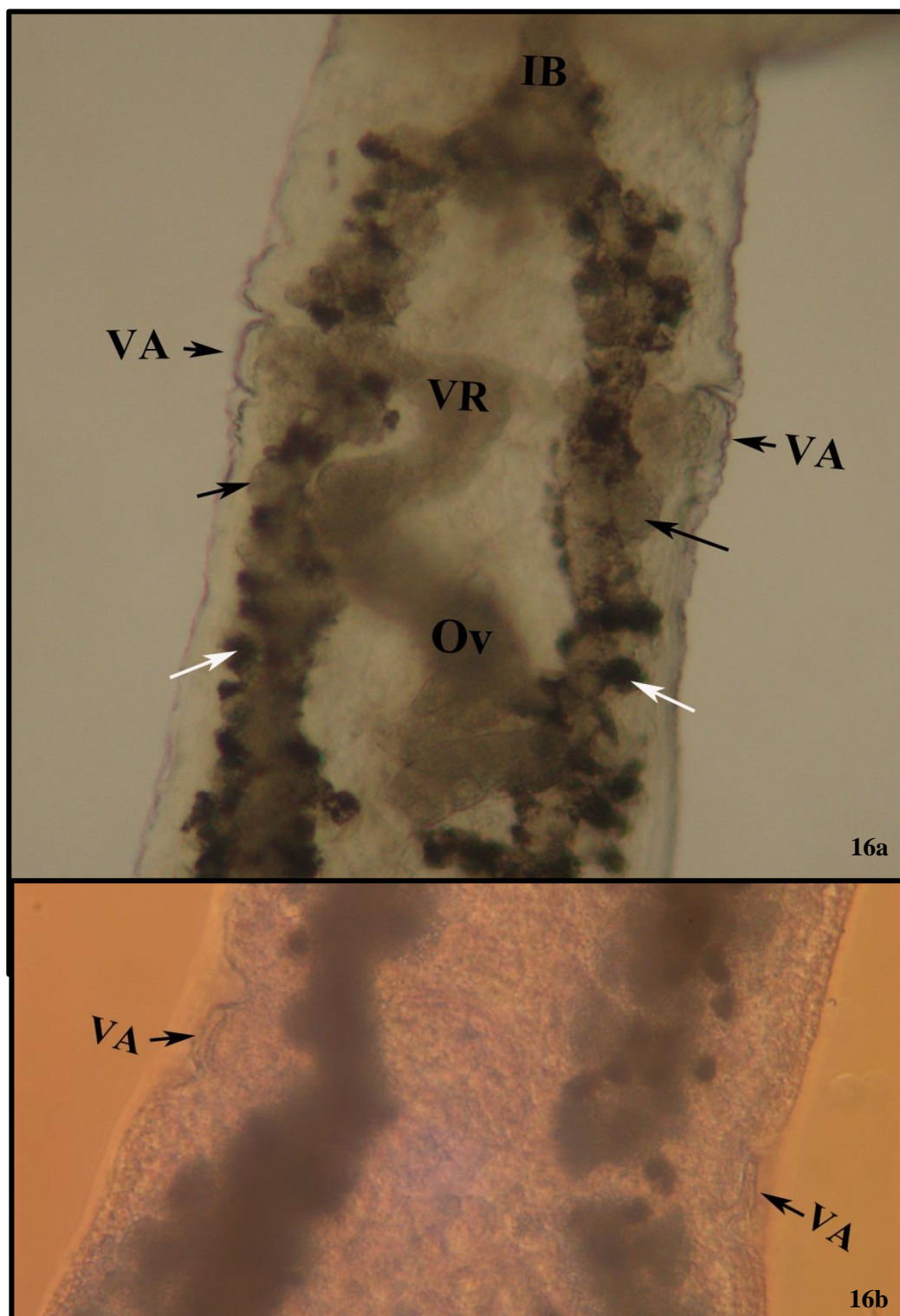
**Plate 14. Genus N., sp. n.** new material collected from the Atlantic seabream *Archosargus rhomboidalis*. **a, g.** complete body; **b-e** anterior end showing the oral suckers (OS); **f.** this picture shows the passage of food from the oral cavity (OC) directly to the esophagus (E) and later to the intestinal cecae which initiate from the intestinal bifurcation (IB); **g.** this shows the extend of the intestinal cecae and their rejoin prior to arrive the peduncle (Pe) in which continue as a single branch.



**Plate 15. Genus N. sp. n.- a.** single dorsal branch of the intestine reaching the opisthaptor; **b-e** spines of male copulatory organ observed from different focus

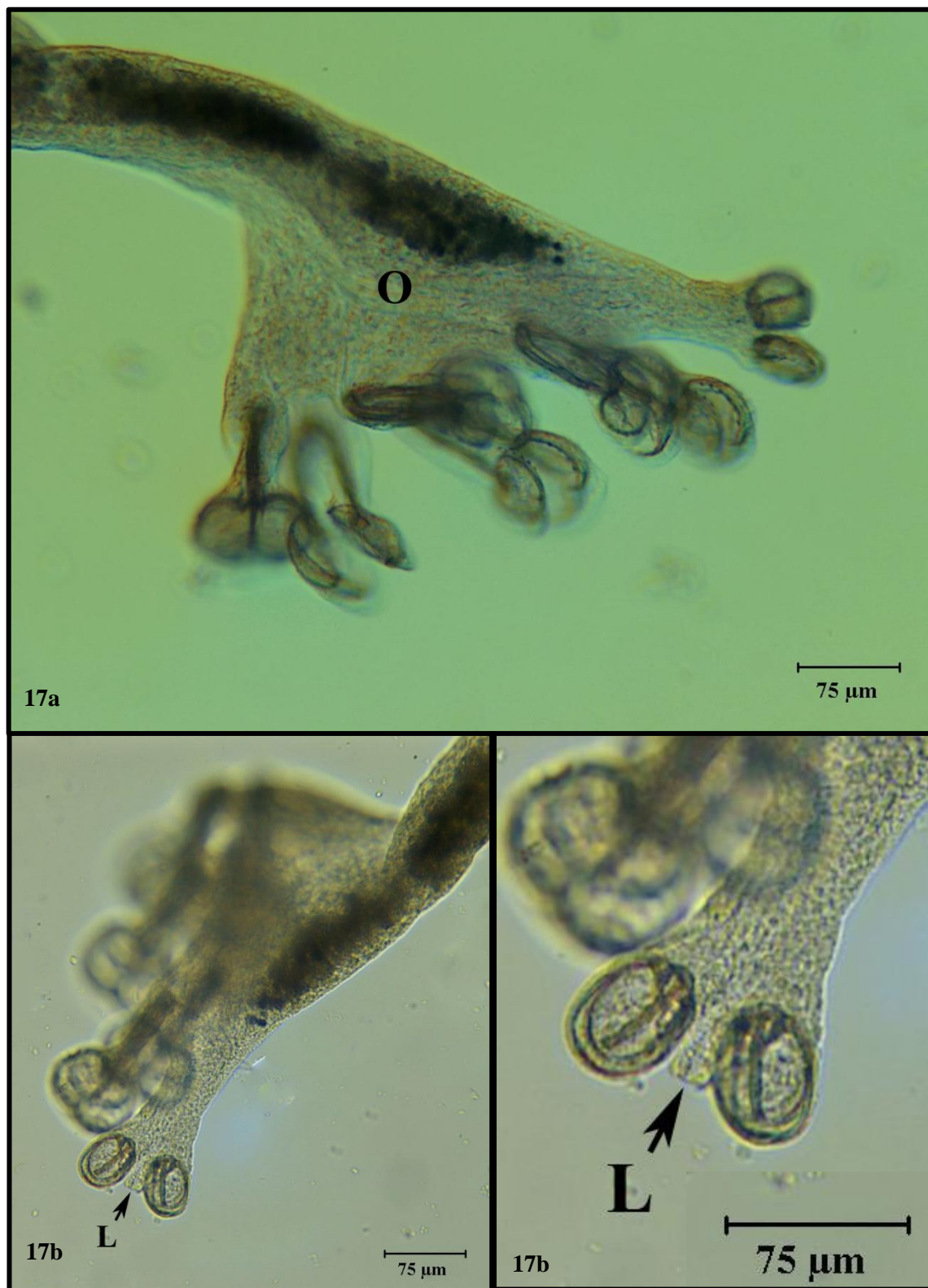


**Plate 16. Genus N. sp. n.-** Female Reproductive System and/or genitalia. **a.** is pointed the vaginal aperture (VA) at both ventrolateral sides at level of the vitelline reservoir, this is connected to the ovary (O). the black arrow is pointing the vitellaria while the white arrows are pointing the blood (food) in the intestinal cecae; **b.** in this picture is pointed the lids that are covering the vaginal aperture found ventrolaterally.



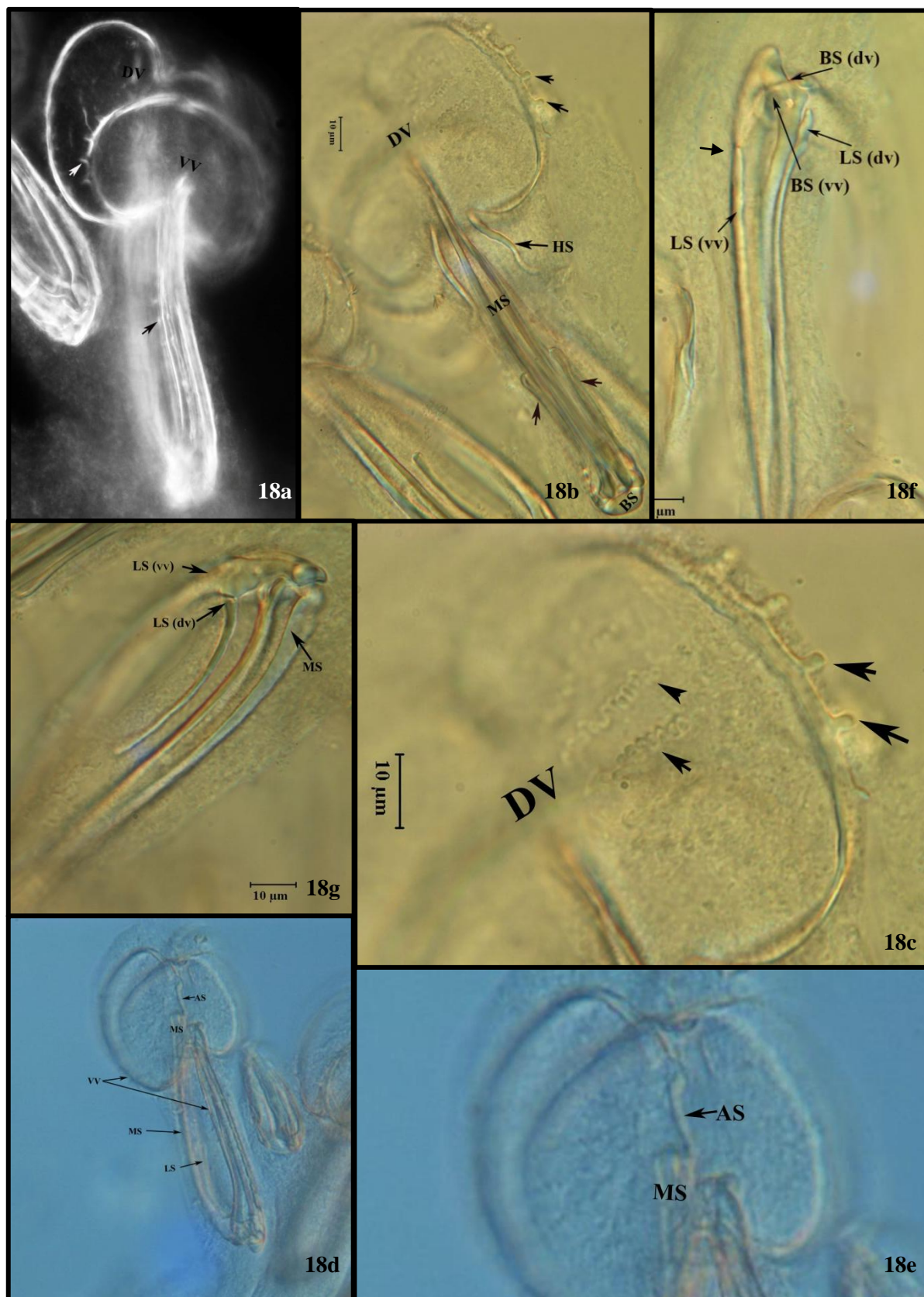
**Plate 17. Genus N. sp. n.- a.** opisthaptor (O); **b-c.** opisthaptor showing the last and small pair of clamps with the lappet between them.



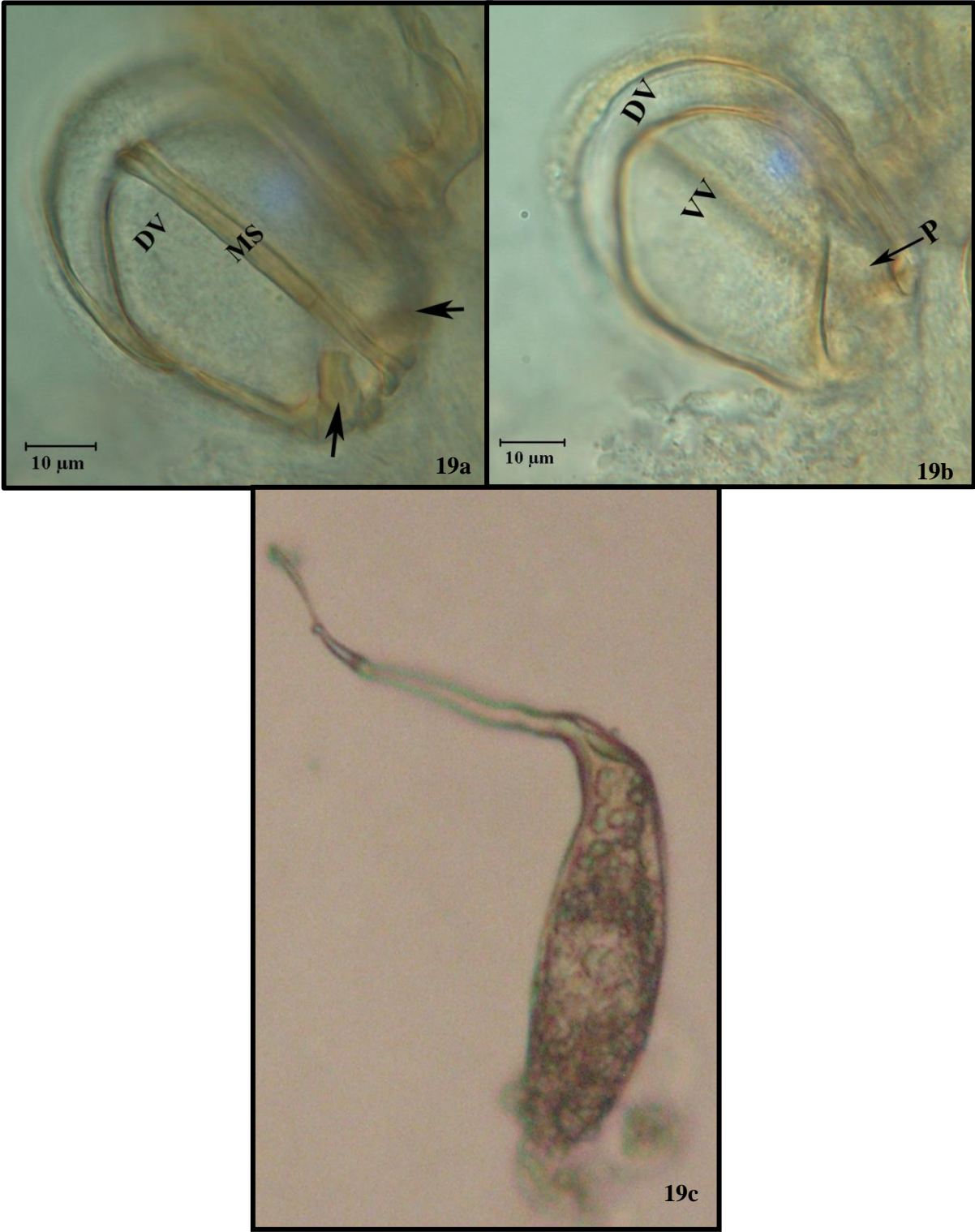




**Plate 18. Genus N. sp.n.-** **a.** large clamps (both valves, dorsal and ventral); **b.** dorsal valve (DV), short spines around the distal segment of the lateral sclerite, hanging sclerite (HS), median sclerite (MS) and are pointed the proximal segments of lateral sclerites; **c.** short spines in the lateral sides of distal segment of dorsal valve and the rows of bars of this wide segment; **e.** accessory sclerite (AS) of median sclerite; **f.** outer view of ventral valve, is observed the fusion of the basal sclerite (BS) with the lateral sclerite of ventral valve in one side and their articulation with the other side. Also is observed both ends of the basal sclerite the ones oriented toward the dorsal valve and the ones oriented toward the ventral valve; **g.** articulation of the median sclerite and proximal segments of dorsal valve with the basal sclerite fused to the lateral sclerite of ventral valve.



**Plate 19. Genus N. sp. n. – a-b.** dorsal and ventral valves respectively, the abbreviations corresponds to dorsal valve, (DV), ventral valve (VV), median sclerite (MS) and plate (P). the arrows in the picture a corresponds to the ventral valve accessory sclerite which are onoriented toward the other side and are visible with the dorsal valve; c. egg.

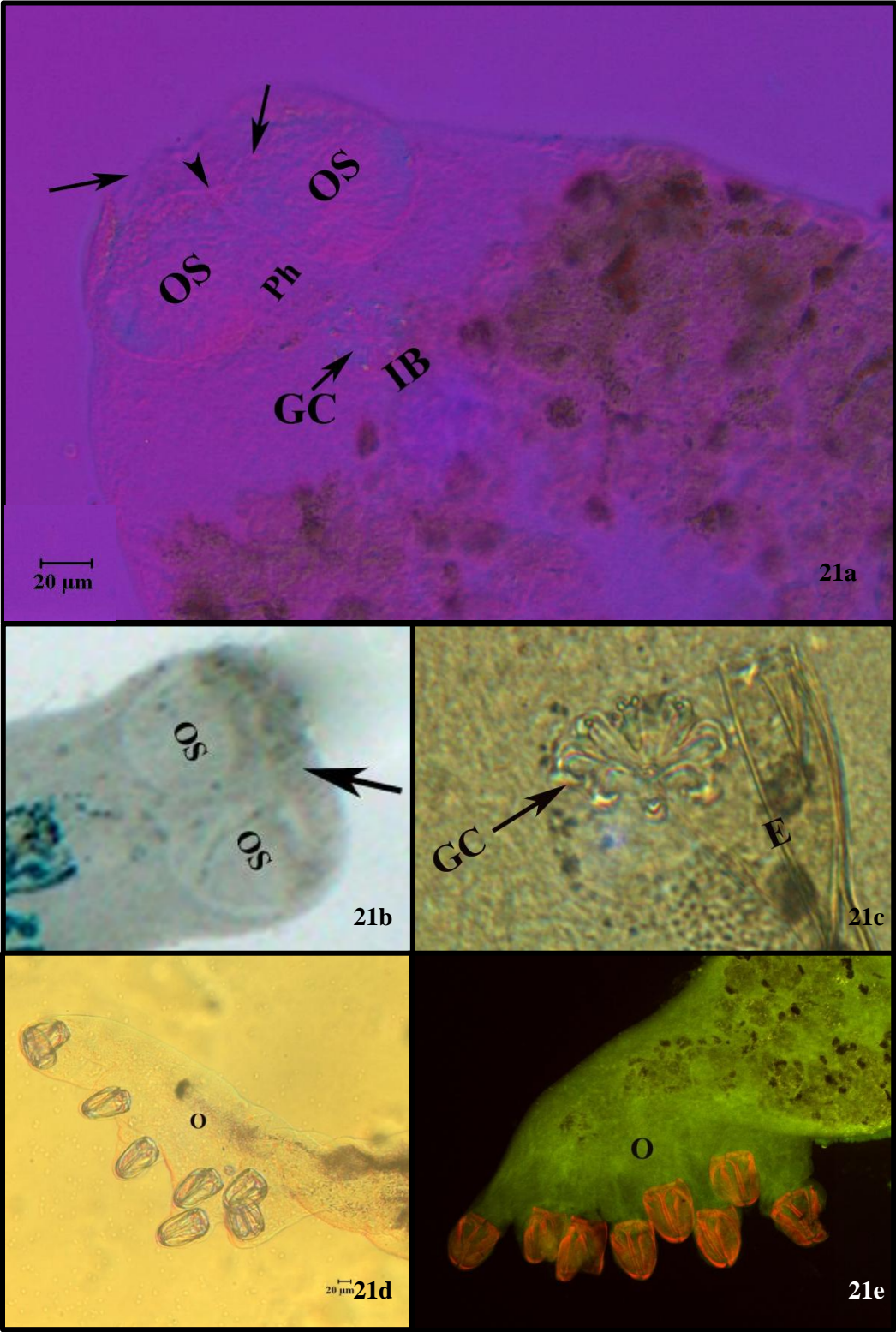


**Plate 20.** *Pseudotagia* **sp. n.** New material collected from the roughneck grunt *Pomadasys corvinaeformis*. **a-b.** body complete



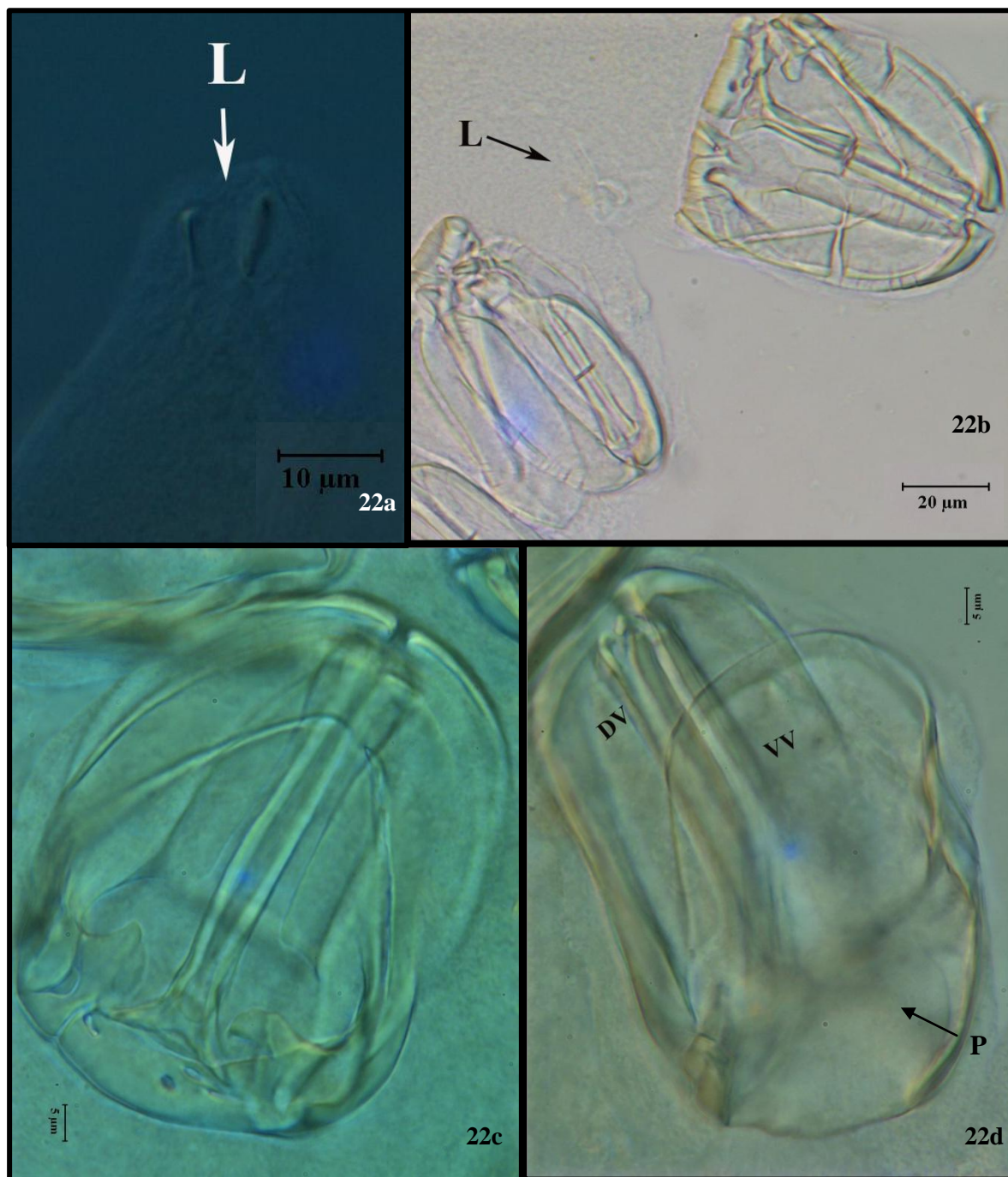


**Plate 21. *Pseudotagia* sp. n.** – **a.** anterior end showing the oral suckers (OS), pharynx (Ph) and is pointed the glands around the oral disc and the entrance to the oral cavity, also is shown the genital corona of spines of the male copulatory organ and the intestinal bifurcation. **b.** glands around the oral disc; **c.** genital corona of spines of the male copulatory organ and one egg at the side; **d-e.** opisthaptor (O) with Nomarsky and confocal microscopy respectively.

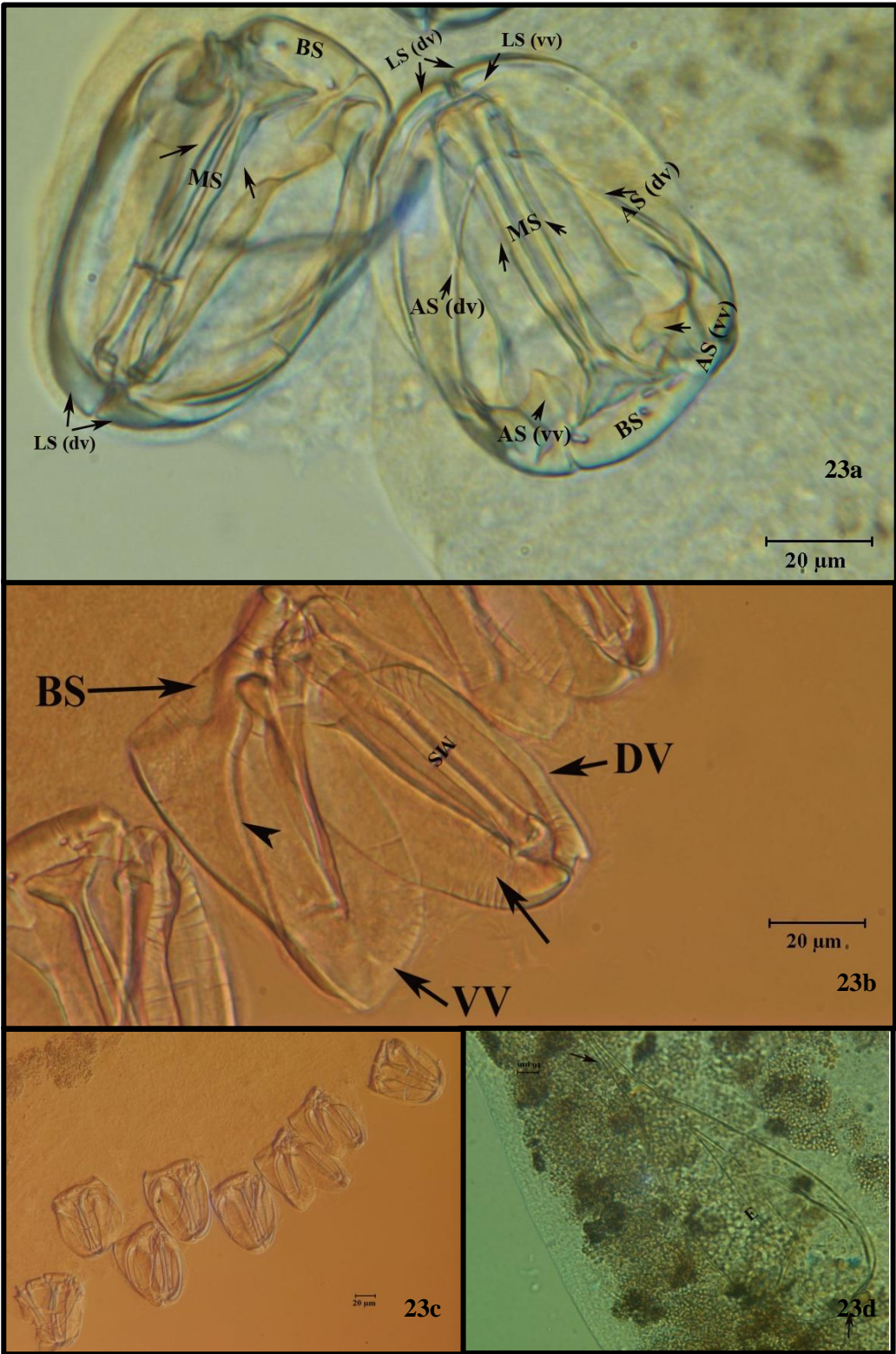




**Plate 22.** *Pseudotagia* sp. n. – **a-b.** lappet (L) with two pair of hooks; **c.** clamp closed; **d.** clamp open distinguishing from the two valves.



**Plate 23. *Pseudotagia* sp. n.** – **a.** clamps showing the sclerite of both valves, the arrows in the left clamp are pointing the distal end of the lateral sclerite in the dorsal valve and the others the reinforcement accessory of the median sclerite of the dorsal valve. The right clamp are pointing also the distal end of the lateral sclerite of dorsal valve, the distal end of the lateral sclerite of ventral valve (LS (vv)), at midline area are pointing at each side of the median sclerite the reinforcement accessory of this sclerite, in both sides are pointing the accessory sclerites of dorsal valve, and at both sides of the basal region are pointed the accessory sclerite of the ventral valve.; **b.** open clamp, showing both valves by separated and the complete side view of the basal sclerite (BS), in the dorsal valve (DV) is pointed the plate that covers the lateral sclerite and part of the outer part of the dorsal valve; **c.** the eight clamps showing that the outer clamps are smaller than the inner ones; egg inside the uterus pointing the round pole and the fusiform one.



### Chapter III.

#### Phylogeny and Revision of the family Macrovalvitrematidae Yamaguti, 1963 (Monogenea: Heteronchoinea: Oligonchoinea)

**Abstract:** The revised classification of Boeger and Kritsky (1993, 1997 and 2001) incorporated the Macrovalvitrematidae into the family Diclidophoridae since recognition of the former family would confer paraphyletic status to the Diclidophoridae. The phylogenetic position of this group has not been resolved nor has the relationship among the members of the family. In this study the genera and species recognized by Bravo-Hollis (1982) as members of the family Macrovalvitrematidae were examined. In addition, the study includes the two genera and species (*Papillopseudotagia hubbsi* and *Pseudohargisia cortesi*) placed in the family previously by Payne (1987). The monophyly of the family Macrovalvitrematidae was tested based on morphological characters using parsimony and bayesian phylogenetic methods. The matrix was created with WinClada 1.00.08 (Nixon, 1999-2002). The software Nona (Goloboff, 1993) and PAUP\* 4.0 WIN 32/Dos Beta Version 10 (Swofford, 2001) were used for the parsimony analysis and Bayesian analysis was performed with the software MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2003). All the 42 characters used for the analyses are presented with a discussion about the evolution of the character states and resulted in a completely resolved tree (length=85 steps; consistency index= 84%; retention index =88%; homoplasy index=15%) suggesting the monophyly of the Family Macrovalvitrematidae and four synapomorphies. The analysis also propose the paraphyly of Diclidophoridae. The topology of the three cladograms with the highest posterior probabilities resulting from the Bayesian analysis is similar to the hypothesis resulting from the parsimony analysis, suggesting the monophyly of the Macrovalvitrematidae, the basal position for the genus *Pseudotagia* and the paraphyletic status of the Diclidophoridae is

preserved. A revised classification for the Family Macrovalvitrematidae is proposed based on the results of the phylogenetic analyses, the analysis of the character transformations along the cladogram; and other morphological characters not analyzed but are discussed. The revised classification proposed for the family Macrovalvitrematidae Yamaguti, 1963 includes: the recognition of nine genera and 14 species, including one new genus and species and one new species of *Pseudotagia* from Puerto Rico; the recognition of the two previously erected subfamilies and the proposal of two new ones to include the members of *Pseudotagia* and for *Papillopseudotagia* respectively; and the reassignment of the genera *Neomacrovalvitrema* Suriano, 1975 and *Pseudohargisia* Payne, 1987 to the genera *Macrovalvitrema* Caballero y C. and Bravo-Hollis, 1955 and *Nicolasia* Suriano, 1975.

## Introduction

The Class Monogenea van Beneden, 1858 represent a diverse group of parasitic platyhelminthes with approximately 720 genera distributed in 53 families (Boeger and Kritsky, 1993) that live mostly on the gills and external surfaces of marine and freshwater fishes. The principal morphological features with important taxonomic importance are the adhesive structures of the anterior end, the genitalia and the attachment organs of the opisthaptor. Many feed on blood and are found attached to the highly vascularised gill tissues of their hosts.

The Monogenea are an ideal group for study of diversification and diversity for at least three reasons: they are diverse both in terms of morphology and number of species, they are generally host specific, and their phylogeny is well resolved, at least to the family level (Poulin, 2002). Poulin (2002) also observed that monogeneans are an excellent group to study because their host specificity allows evolutionary trends to be inferred. The most accepted classification of the family (by Boeger and Kritsky 1993, 1997, 2001) who proposed two subclasses Polyonchoinea and Heteronchoinea. In their classification the Heteronchoinea is divided into two infrasubclasses, Poystomatoinea and Oligonchoinea. The Polystomatoinea parasitize amphibians while the Oligonchoinea only are found in fishes. The members of the subclass Heteronchoinea have oral suckers, a genitointestinal canal and an opisthaptor armed with clamps or suckers and larval hooklets persisting from the oncomiracidium (larval stage) Hernández-Vale (2003).

Whether the class of Platyhelminthes comprising the monogenean flatworms should be referred to as Monogenea or Monogenoidea has been debated since the early part of the 20<sup>th</sup> century (Wheeler and Chisholm, 1995). In this study the class is referred to as Monogenea van Beneden 1858 based in the resolution of the ICOPA Round Table (summarized by Euzet and Prost, 1981)

which considered that in the absence of defined rules, names for higher taxa should be based on a combination of priority, stability and consensus among specialists, with stability and consensus the most important considerations (Wheeler and Chishom, 1995).

The phylogenetic relationships among monogenean subclasses and families have been controversial with several competing hypotheses (Boeger and Kritsky, 2001) conferring either a monophyletic or a polyphyletic status to the class. Those considering Monogenea as monophyletic group are based on morphological analyses by Ehlers (1985); Brooks (1989; and by Boeger and Kritsky (1993, 1997 and 2001). Boeger and Kritsky (1993) listed several synapomorphies but Mollaret (1997) rejected their monophyly based on molecular analysis. Studies of sperm structure by Justine (1991, 1993 and 2001), found no synapomorphy for the Monogenea. Mollaret et al. (2000) suggested paraphyly of the Monogenea and monophyly of the subclasses Monopisthocotylea (Polyonchoinea) and Polyopisthocotylea (Heteronchoinea). Littlewood and Olson (2001) also failed to support monophyly, but in 2002 Olson and Littlewood presented molecular data that strongly support monophyly of the class. Boeger and Kritsky (1993) argued that molecular studies are based on only a few taxa which did not represent the real composition of the class. The phylogenetic status of the subclasses (Polyonchoinea and Heteronchoinea) has been consistent in suggesting the monophyly for each subclass.

The revised classification of Boeger and Kritsky (1993, 1997 and 2001) incorporated the Macrovalvitrematidae into the family Diclidophoridae since recognition of the former family would confer paraphyletic status to the Diclidophoridae. According to the evolutionary



hypothesis of Mamaev (1976), the Macrovalvitrematidae have their origins within the Diclidophoridae and share common ancestors with diclidophorid genus (Boeger and Kritsky, 1993). Diclidophorids are considered a phylogenetically ancient group, one of the most predominant families of deep-sea monogeneans (Justine et al., 1995, Rohde, 1988).

Yamaguti, 1963 erected the family Macrovalvitrematidae and recognized 10 valid genera and 12 species distributed among the pacific coast of Mexico, California, Perú; the south Atlantic coast of Argentina and Brazil and the Gulf of Mexico and the southeastern coast of United States (North Atlantic). The first part of this study was dedicated to determine the macrovalvitrematid fauna in fishes of Puerto Rico resulting in the report of new locality records for two species and the proposal of two new species and one new genus as member of Macrovalvitrematidae (see chapter I). These new species and new genus have been included in the phylogenetic analysis realized in this chapter.

In addition to be unresolved the phylogenetic position of the Macrovalvitrematidae the relationships among the member has not been analyzed using a cladistic criteria. The classification of Bravo-Hollis (1982) is the most recent (Table III1) and this separated the genera reported until that moment into two subfamilies based in the spines of the male copulatory organ and the clamp composition of the opisthaptor. Another aspect of interest is that most of the genera are monotypic with the only exception of *Pseudotagia*. The phylogenetic analysis can help to address the relation among the members of the family and to suggest possible new combinations. One new combination, *Macrovalvitrema argentinensis* n. comb. (Suriano, 1975) is already suggested in the first chapter and this phylogenetic analysis could support or reject it. In

this chapter this species will be recognized as their original combination *Neomacrovalvitrema argentinensis* Suriano, 1975.

This work represents a morphologic comparative study among the taxa belonging to the Macrovalvitrematidae and is proposed the first phylogenetic hypothesis for 10 of their 11 genera and nine of the 12 species of the family and proposed new taxonomic classification based on the obtained results. The phylogenetic hypotheses were performed using parsimony and bayesian analysis.

## Review of Literature

### I. Historic Revision of the Family

The first macrovalvitrematid described was *Tagia micropogoni* by Pearse (1949) and redescribed by Hargis (1956), who also assigned to the genus *Tagia* the species *T. bairdiella* and *T. cupida* all from the north Atlantic. Caballero y C. and Bravo-Hollis (1955) described the species *Macrovalvitrema sinaloense* and *Pterinotrematoides mexicanum* from the pacific coast of Mexico. Yamaguti (1963) erect the family Macrovalvitrematidae and assigned to it those five species, maintaining the original names of the pacific species but creating new combinations for the species of the north Atlantic. He erected three new genera and assigned *Tagia micropogoni*, *T. bairdiella* and *T. cupida* to *Macrovalvitrematoides micropogoni*, *Hargisia bairdiella* and *Pseudotagia cupida* respectively. Yamaguti classify those five genera based on the vagina.

Crane (1972) described *Pseudotagia clathratus* from the pacific coast of California. Suriano (1975) add to the family three new genera and three new species (*Neomacrovalvitrema argentinensis*, *Neopterinotrematoides avaginata* and *Nicolasia canosorum*) from the south Atlantic coast of Argentina. Bravo-Hollis, (1982) classify those eight genera and nine species into two subfamilies Macrovalvitrematoidinae and Pterinotrematoidinae based in the opisthaptor clamps arrangement and the spines of the male copulatory organ. Their classification related the genera *Pterinotrematoides*, *Neopterinotrematoides*, *Hargisia* and *Nicolasia* in the subfamily Pterinotremaotidinae and the genera *Macrovalvitrema*, *Macrovalvitrematoides*, *Neomacrovalvitrema* and *Pseudotagia* in the Macrovalvitrematoidinae.

Payne (1987) adds two more new genera and new species, *Pseudohargisia cortesi* and *Papillopseudotagia hubbsi* and assigned respectively to the subfamily Pterinotrematoidinae and Macrovalvitrematoidinae. Luque et al. (1993) described the last valid species included in the family, *Pseudotagia rubri* from south Atlantic coast of Brazil. Fuentes Zambrano et al. (2009) reported as *Pterinotrematoides mexicanum* specimens that coincide with the specimens proposed in this study as a new genus and species of Macrovalvitrematidae.

## II. Phylogenetic position and studies

Mamaev (1976) suggests that the Macrovalvitrematidae has their origin within the Diclidophoridae. Mamaev and Lebedev (1979) located the Macrovalvitrematidae in the suborder Discocotylinea. Lebedev (1988) maintains the Macrovalvitrematidae in the Discocotylinea but together with the Diclidophoridae and Anthocotylidae, along with Discocotylidae, Diplozoidae and Octomacridae. The analysis of Boeger and Kritsky (1993) does not support the inclusion of the former families in the suborder Discocotylinea and proposed the transfer of the Diclidophoridae to the suborder Microcotylinea. Boeger and Kritsky (1993) incorporated and tentatively consider the Macrovalvitrematidae synonyms of Diclidophoridae based on Mamaev's (1976) phylogenetic hypothesis for the Diclidophoridae. Pérez-Ponce de León (1997) use the macrovalvitrematid species *Macrovalvitrema sinaloense* and *Pterinotrematoides mexicanum* as an outgroup in the phylogenetic analysis of the family Pterinotrematidae Bychowsky & Nagibina, 1959. Boeger and Kritsky did not recognized the family Macrovalvitrematidae because their apparent origins within the Diclidophoridae.

**Table III-1. System of classifications for the Family Macrovalvitrematidae Yamaguti, 1963 proposed by various authors****A. Yamaguti, 1963**

Superfamily Diclidophoroidea Price, 1936

Family Macrovalvitrematidae

Genera included:

*Macrovalvitrema* Caballero y C. and BravoHollis, 1955 (**Type genus**)*Pterinotrematoides* Caballero y C. and BravoHollis, 1955*Macrovalvitrematoides* Yamaguti, 1963*Hargisia* Yamaguti, 1963*Pseudotagia* Yamaguti, 1963**B. Mamaev and Lebedev, 1979**

Order Mazocraeidea Bychowsky, 1957

Suborder Discocotylinea Bychowsky, 1957

Family Macrovalvitrematidae Yamaguti, 1963

**C. Bravo-Hollis, 1982**

Order Mazocraeiformes Caballero y Bravo, 1961

Suborder Discocotylinea Bychowsky, 1957

Family Macrovalvitrematidae Yamaguti, 1963

Subfamily Macrovalvitrematoidinae Bravo-Hollis, 1982

Genera included:

*Macrovalvitrema* Caballero y C. and Bravo-Hollis 1955 (**Subfamily type genus**)*M. sinaloense* Caballero y C. and Bravo-Hollis, 1955*Macrovalvitrematoides* Caballero y C. and Bravo-Hollis, 1955*M. micropogoni* Pearse, 1949*Neomacrovalvtirema* Suriano, 1975*N. argentinensis* Suriano, 1975*Pseudotagia* Yamaguti, 1963*P. cupida* Hargis, 1956

Subfamily Pterinotrematoidinae Bravo-Hollis, 1982

Genera included:

*Pterinotrematoides* Caballero y C. and Bravo-Hollis, 1955 (**Subfamily type genus**)*P. mexicanum* Caballero y C. and Bravo-Hollis, 1955*Hargisia* Yamaguti, 1963*H. bairdiella* Hargis, 1956*Neopterinotrematoides* Suriano, 1975*N. avaginata* Suriano, 1975*Nicolasia* Suriano, 1975*N. canosorum* Suriano, 1975

## Material and Methods

### Taxa Studied

In this study the genera and species studied include those recognized by Bravo-Hollis (1982); the two genera and species described by Payne (1987) (*Papillopseudotagia hubbsi* placed in Macrovalvitrematinae and *Pseudohargisia cortesi* in the Pterinotrematoidinae); *Pseudotagia rubri* described by Luque et al. (1993); and the new material collected from Puerto Rico including one new species referred to as *Pseudotagia* sp. A and new Genus referred to as Genus A. Table III2 includes the taxa analyzed. The type specimens of *Hargisia bairdiella* (Hargis, 1956), *Pseudotagia cupida* (Hargis, 1956) and *Pseudotagia clathratus* were not available for the study. The first two have been lost in previous loan and the last the material was in very poor condition to establish the character states of each structure analyzed as a character.

The monophyly of the family Macrovalvitrematidae was tested based in the analysis of the characters that allow distinguishing them from the other monogeneans. The character states were based in the recognition of primary homology among the structures used as a character. The primary homologies of these characters were tested using parsimony and bayesian phylogenetic analyses.

### Phylogenetic Analysis

This study presents a hypothesis about the phylogenetic relationships of the genera and species of the family Macrovalvitrematidae using the cladistic method. The characters and the character states were obtained from the examination of new material collected, type specimens deposited in museums and microphotographs of some type specimens not available for loan and provided

by the US National Parasite Collection (Table III2). Sixteen taxa were used for the cladistic analysis, five representing the outgroup and 11 representing the ingroup (Table III3). The family Macrovalvitrematidae is composed mostly of monotypic genera. The characters for those genera were determined by an analysis of the specimens available to study. The only genus not represented in this analysis was *Hargisia bairdiella*. Two of the four species of the genus *Pseudotagia* were also not available (*P. cupida* Hargis, 1956; and *P. clathratus* 1972). The remaining two species analyzed in this study were *P. rubri* Luque et al, 1993 and 49 specimens of a new species described in Chapter I of this work, denominated *Pseudotagia* sp A, collected from the west (Caribbean) and north (Atlantic) coasts of Puerto Rico. Is also included in the analysis is the information from 25 specimens of a new genus and species also described in Chapter I, collected from the west coast of Puerto Rico was and will be recognized in the hypotheses as Genus A. Some potential characters were not included in the analysis because the absence of information and some uncertain characters which could, by misinterpretation, obscure the relationships among the taxa studied. This will be discussed at the end of the description of the characters and their states.

The analysis considered 43 characters, including binary and multistated character states and the use of Fitch optimization (non-additive) for all of them. Undetermined character states were defined as (?) and the taxa for which a character did not apply were designated as (-).

### **Outgroup Selection**

The outgroups used for the analysis include members of families more related to the Macrovalvitrematidae according to the Boeger and Kritsky (1993) hypothesis. This includes

members of the families Allopyrographoridae Yamaguti, 1963; Pyragraphoridae and Diclidophoridae. The outgroup taxa includes vouchers of *Allopyragraphorus hippos* (Hargis, 1956) and *Pyragraphorus pyragraphorus* (MacCallum et MacCallum, 1913), and three diclidophorids each one representing a different subfamily of Diclidophoridae. *Eurysorchis* sp., (Subfamily Eurysorchiinae Yamaguti, 1963); *Choricotyle* sp. (Choricotylinae Sproston, 1946) and *Mamaevicotyle villalobosi* (Diclidophorinae Cerfontaine, 1895). All the diclidophorids used were collected from Puerto Rico as part of the study, except *M. villalobosi* for which two paratype specimens were examined. Three specimens of genus *Pedocotyle* MacCallum, 1913 were also collected, representing another subfamily of Diclidophoridae, (Pedocotylinae Yamaguti, 1963). But these were not included in the analysis because it was difficult to apply primary homologies to this genus. According to Bychowsky (1957) this genus stands somewhat apart from the rest of the diclidophorids, apparently representing a deviation from the general type of development of diclidophorids and, in a certain measure, its structure is interesting from the point of view of convergency with Hexabothriidae.

## Matrix

The matrix (Table IV) was constructed using the program WinClada 1.00.08 (Nixon, 1999-2002) and afterward a nexus file was created to analyze the characters in PAUP\*. Also the nexus file was modified in wordpad to submit in MrBayes v3.1.2 for the Bayesian analysis. To run the parsimony analysis in Paup\* and the bayesian analysis with Mrbayes v3.1.2 the inapplicable characters were assigned in the corresponding nexus file as (?) to be recognized by the programs.



### **Parsimony Analysis**

The parsimony analysis and the reconstruction of the characters were done using the programs Nona (Goloboff, 1993) and PAUP\* 4.0 WIN 32/Dos Beta Version 10 (Swofford, 2001). The analyses were made using mult 20 and mult 50, heuristic analysis with 100 replicates (hold max tree= 100; Mult\*100; hold/ 2' Multiple TBR+ TBR multi\*Max for Nona; hsearch addseq=random nreps=1000 hold=2 maxtree=100 for PAUP\* 4.0 beta version 10. The character examination in the most parsimonious cladograms was made using WinClada. Both optimizations ACCTRAN (fast optimization) and DELTRAN (slow optimization) in WinClada were used to analyze the multiples changes in character states. The values of bootstraps (bootstrap nreplicates= 1000 search=heuristic/addseq=random) and strict consensus were considered as measure of support of the strong clades that resulted. Although the autapomorphic characters could inflate the consistency index (CI) value, these were maintained to support the diagnoses of the genus and species examined.

### **Bayesian Analysis:**

The Bayesian analysis was made using the program Mrbayes v3.1.2 (Huelsenbeck and Ronquist, 2003). To obtain the estimates of the posterior distribution of trees was performed the technique of MCMC (Markov Chain Monte Carlo). As mentioned above the matrix was modified to the requisits of the Mrbayes, where it and was necessary to specify the datatype as “standard” referring to morphological data. Before performing the analysis, some parameters were defined. These included the selection of an evolutionary model, specification of prior distributions and

specification of parameters to control the MCMC algorithm. The analysis was performed according to Lewis (2001) and Glenner et al. (2004). The command `lset` was used to set the parameters of the likelihood model. The data (morphological) was run under the datatype “standard” option that activates the M2 (MK) evolutionary model Mk +Gamma. The rates of the model, was changed to gamma using ‘`lset rates=gamma`’. The model settings include data type= standard; coding= variable; number of states= variable; and the rates= gamma. The following parameters were set for the prior distribution: (1) state frequency (`statefreqpr`), (2) shape (`shapepr`), (3) topology (`topologypr`) and (4) branch length (`Brlenspr`) using the command `prset`. These were set as following: “`prset statefreqpr = dirichlet 1 shapepr=uniform topologypr=uniform brlenspr=unconstrained:exponential 10.0`”. Finally the MCMC algorithm was set using the `mcmcp` command performing 10,000 iterations and collect every 100<sup>th</sup> value and run four chains in parallel (three heated chains and one “cold” chain as stated by default in the program. To set these values was typed: “`mcmcp ngen=10000 nchains=4 printfreq=100 samplefreq=100`”. The resulting tree was observed with TreeView (Page, 2001). The analysis was summarized using the commands “`sumt`” and “`sump`”. The cladogram and the clade credibility values for the nodes were compared with the cladogram and the bootstrap values resulted from the parsimony analysis.

**Table III-2** List of Monogenean Species examined for the Taxonomic Study and Phylogenetic Analysis. US National Parasite Collection, Beltsville, Maryland, USA (USNPC); Colección Nacional de Helminths, Universidad Autónoma de México (CNHE); Museo de La Plata, Universidad nacional de La Plata, Argentina (MLP) and Harold W. Manter Laboratory University of Nebraska State Museum, Lincoln, Nebraska, USA (HWML)

	Monogenean Species	No specimens examined	Museum Number	Host Species
I N G R O U P	<b>Family Macrovalvitrematidae</b>			
	<i>Macrovalvitrematoides micropogoni</i>	3	USNPC No. 36961*;	<i>Micropogonias undulatus</i>
	<i>Macrovalvitrema sinaloense</i>	6	CNHE No. 213-215 (314) 1 paratype CNHE No. 231-6 (102) 1 voucher CNHE No. 231-10 (101) 4 vouchers	<i>Micropogonias ectenes</i> <i>M. megalops</i> <i>Lutjanus argentiventris</i>
	<i>Pterinotrematoides mexicanum</i>	2	CNHE No. 213-216 (098) paratypes	<i>Micropogonias ectenes</i>
	<i>Pseudotagia clathratus</i>	2	USNPC No. 71930- 71931	<i>Paralabrax clathratus</i>
	<i>Pseudotagia rubri</i>	2	USNPC No. 83186-87 paratypes	<i>Orthopristis ruber</i>
	<i>Pseudotagia</i> sp.A	49	New collected specimens	<i>Pomadasys corvinaeformis</i>
	<i>Neomacrovalvitrema argentinensis</i>	1	MLP No. P2 1 holotype	<i>Micropogonias furnieri</i>
	<i>Neopteriotrematoides avaginata</i>	1	MLP No. P3 1 holotype	<i>Micropogonias furnieri</i>
	<i>Nicolasia canosorum</i>	1	MLP No. P4 1 holotype	<i>Umbrina canosai</i>
	<i>Papillopseudotagia hubbsi</i>	2	USNPC No. 79500* (holotype) ; 79501 (1 paratype)	<i>Citharichthys sordidus</i>
	<i>Pseudohargisia cortesi</i>	2	USNPC No. 79499* (holotype) HWML No. 23641 (paratype)	<i>Micropogon megalops</i>
	Genus A	25	New collected specimens	<i>Archosargus rhomboidalis</i>
	<b>Family Allopyrgraphoridae</b>			
	<i>Allopyrgraphorus hippos</i>	5	USNPC No. 85297 (2 vouchers), 85299 (1 voucher) and 85942 (2 vouchers)	<i>Caranx ruber</i> <i>Caranx chrysos</i>
O U T G R O U P	<b>Family Pyrgraphoridae</b>			
	<i>Pyrgraphorus pyrgraphorus</i>	1	HWML 1413 1 voucher	<i>Trachinotus votla</i>
	<b>Family Diclidophoridae</b>			
	<i>Eurysorchis</i> sp.	3	New collected specimens	<i>Pomadasys corvinaeformis</i>
	<i>Choricotyle</i> sp.	3	New collected specimens	<i>Pomadasys corvinaeformis</i>
	<i>Mamaevicotyle villalobosi</i>	2	CNHE No. 235-2 paratypes	<i>Paralabrax maculatofasciatus</i>
	<i>Pedocotyle</i> sp.	3	New collected specimens	<i>Bairdiella ronchous</i>

\* Refers to photos of the holotype material prepared and sent from the United States National Parasite Collection.

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**Tabla III-3. Taxa included in the phylogenetic analysis of the family Macrovalvitrematidae**


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*Macrovalvitrema sinaloense* Caballero y C. and Bravo-Hollis, 1955

*eomacrovalvitremaa argentinensis* (Suriano, 1975)

*Macrovalvitrematoides micropogoni* (Pearse, 1949) Yamaguti, 1963

*Pterinotrematoides mexicanum* Caballero y C. and Bravo-Hollis, 1955

*Neopterinotrematoides avaginata* Suriano, 1975

*Nicolasia canosorum* Suriano, 1975

*Pseudotagia rubri* Luque et al., 1993

*Papillopseudotagia hubbsi* Payne, 1987

*Pseudohargisia cortesi* Payne, 1987

***Pseudotagia* sp. n. A**

**Genus n. A**

*Allopyragraphorus hippos* (Hargis, 1956) Yamaguti, 1963

*Pyragraphorous pyragraphorus* (MacCallum et MacCallum, 1913) Sproston, 1946

*Eurysorchis* Manter et Walling, 1958

*Choricotyle* v. Beneden et Hesse, 1863

*Mamaevicotyle villalobosi* Lamothe Argumedo, 1984

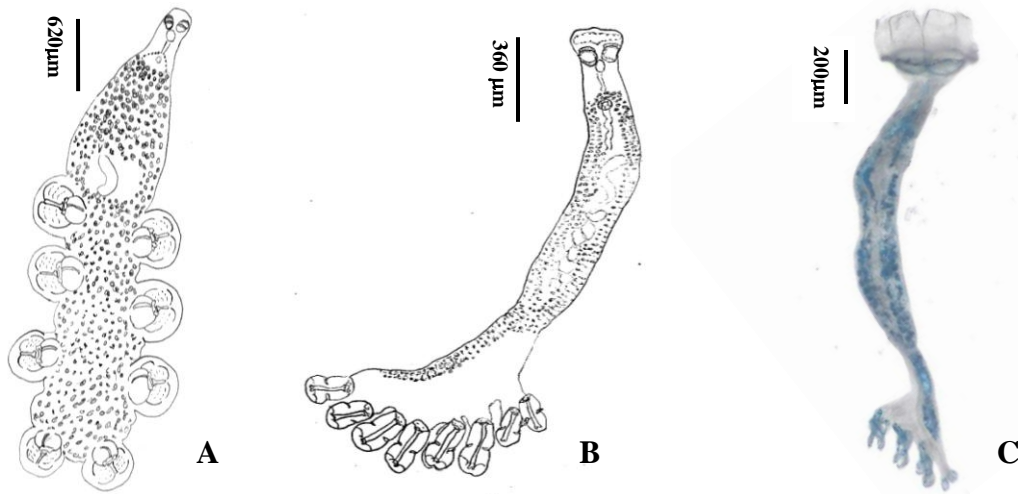
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## Results and Discussion

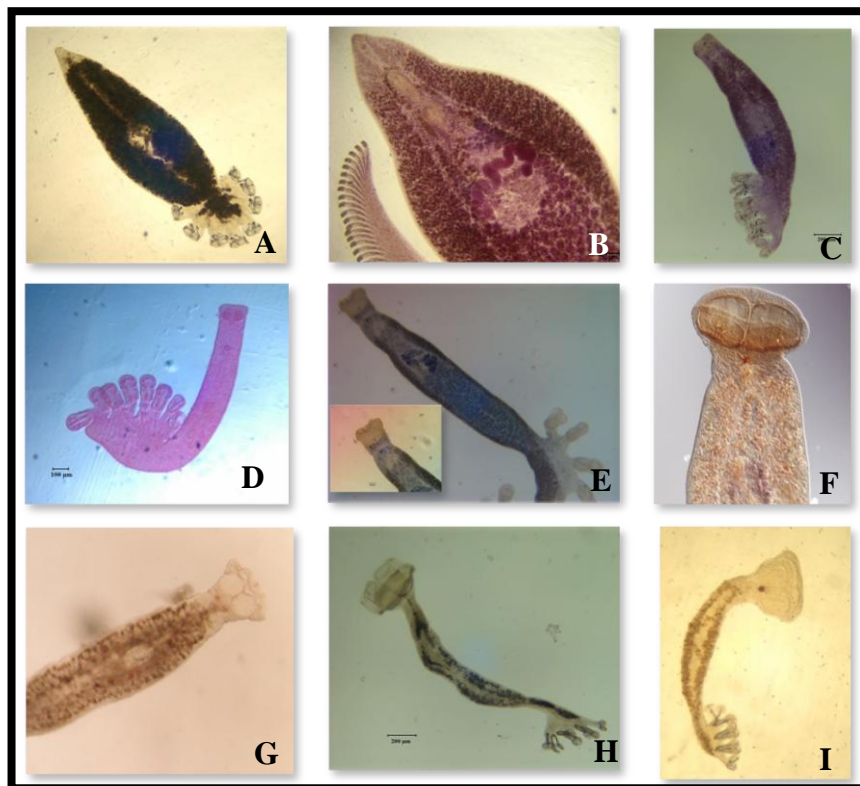
### Parsimony analysis: Character Analysis

In this section the characters used for the parsimony analysis are presented with a discussion about the evolution of the character states. The number in parenthesis refers to the character state according to the coding that appears in the matrix followed by the description of the character state. The number or numbers that appear between the bold brackets [ ] indicate the position of the character state in the cladogram.

1. **Relationship of the Anterior End to the Body Proper: (0)** Tapered anterior end [**1,2,3,4**] (Figure 1A, 2A-B); **(1)** goblet shaped anterior end [**5,6,7,8,9,10,11,12,13,14,15**] (Figures 1B, 2C-G ); **(2)** wide goblet-shaped anterior end (twice as wide as the body proper) [**16**] (Figures 1C, 2H-I). The members of the family Macroalvitrematidae can be distinguished from other monogeneans because their anterior end is goblet-shaped with large oral suckers and a wide oral disc which allows expansion of the oral cavity for the attachment of the oral suckers to the gills of their hosts. The hypothesis resultant from the analysis suggests that the goblet-shape character state is a synapomorphy for the Macroalvitrematidae (Figure II). The character state (2), wide goblet shape is suggested as an autapomorphy for Genus A and also the first report of this kind of development of the anterior end.

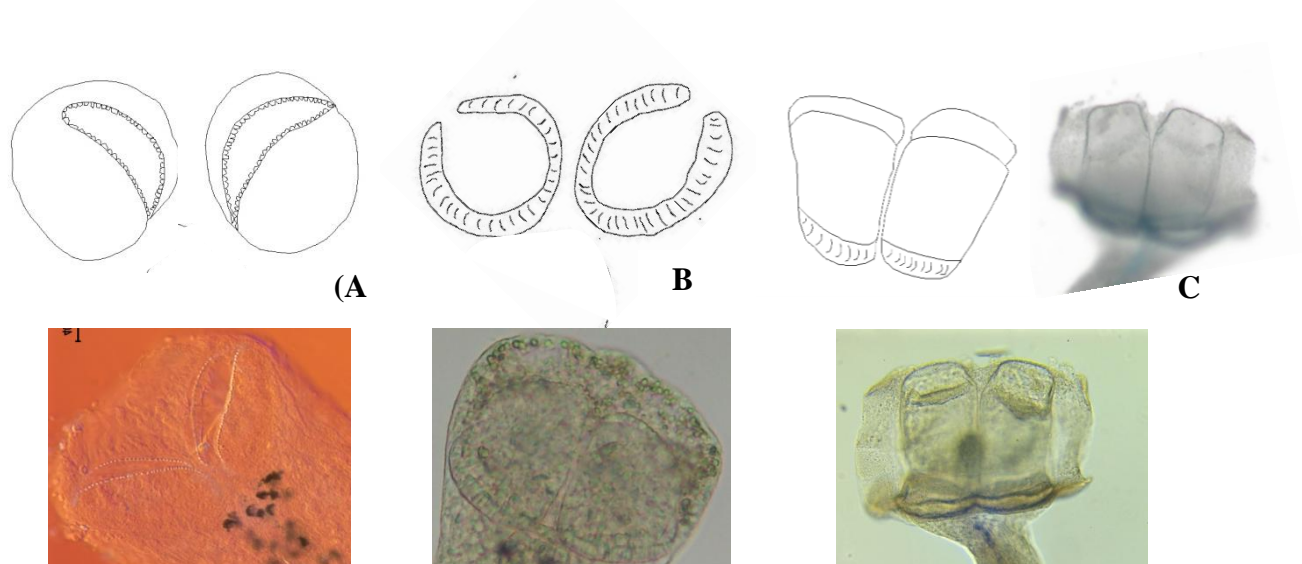


**Figure 1** Character states of the relationship of the anterior end to the body proper. **A** State (0) Tapered anterior end as occurs in diclidophorids; **B**. State (1); Goblet-shaped commonly found in most macrovalvitrematids; **C**. State (2). Wide goblet-shaped observed in one macrovalvitrematid, Genus A



**Figure 2.** Example of the relationship between the anterior end and width of the the body proper among the examined monogeneans. **A-B**. Tapered anterior end of *Choricotyle* sp. and *Allopyragraphorus hippos* respectively; **C-G**. Goblet-shaped anterior end *Pseudohargisia cortesi*, *Macrovalvitrema argentinensis*, *M. sinaloense*, *Papillopseudotagia hubbsi*, and *Neopteriotrematoides avaginata* respectively; **H-I**. Wide goblet-shaped anterior end of **Genus A**, this has the oral disc closed and still is twice as wide as the body proper.

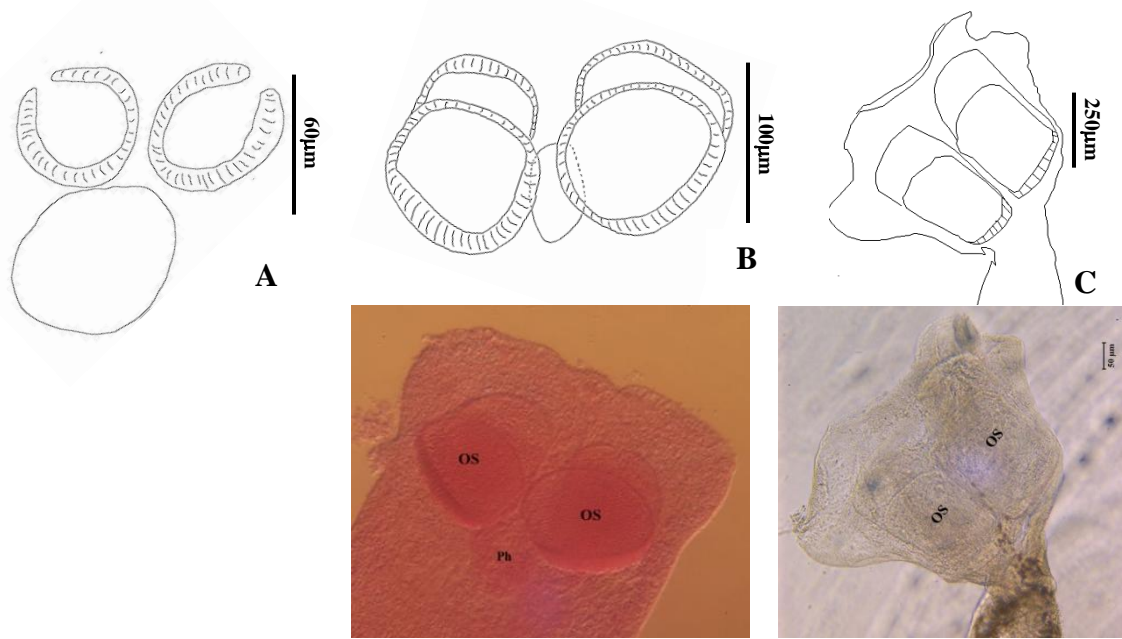
2. **Adhesive reinforcement of oral suckers:** (0) presence of small denticles around the borders of the oral suckers [1,2] (Figure 3A); (1) unarmed and highly muscular [3,4,5,6,7,8,9,10,11,12,13,14,15] (Figure 3B); (2) muscular and slightly sclerotized edges [16] (Figure 3C). The absence of denticles (unarmed), and the presence of muscular suckers, state (1), is apparently a synapomorphy for all the diclidophororids and the macrovalvitrematids examined. The development of large muscular and slightly sclerotized edges in the sucker represents an autapomorphy for the Genus A.



**Figure 3.** Character states of adhesive reinforcement of oral suckers **A. State (0), B. State (1)** and **C. State (2)**

3. **Oral sucker size relative to the pharynx:** (0) oral suckers smaller than pharynx [1,2,3,4] (Figure 4A); (1) larger (more than twice as wide) than pharynx, with dorsal side extending more anteriorly than ventral side [5,6,7,8,9,10,11,12,13,14,15] (Figure 4B); (2) much larger square oral suckers, with dorsal side extending more anteriorly and absence of pharynx [16] (Figure 4C). The decrease in the size of the pharynx as the oral suckers increase in the size is clearly

observed among the macrovalvitrematids. This characteristic corresponds to the character state (1) and is suggested in the hypothesis as a synapomorphy for the macrovalvitrematids with the diclidophorid *Mamaevicotyle villalobosi*. The possession of even larger suckers with a rectangular shape is accompanied by the absence of the pharynx is, according to the hypothesis, an autapomorphy for the **Genus A**. The state (0) is suggested by the hypothesis as the plesiomorphic state, which is maintained in the diclidophorid clade (*Eurysorchis*, *Choricotyle*).



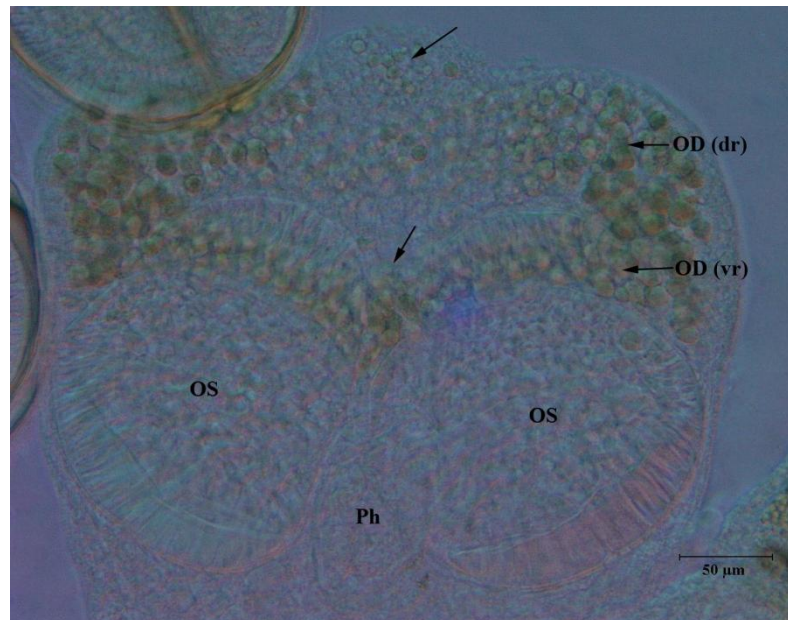
**Figure 4.** Character states for relationship in size between the oral suckers and pharynx. **A. State (0)** *Choricotyle* sp, **B. State (1)** *Macrovalvitrema argentinensis*; **C. State (2)** Genus A .

**4. Glands in border of oral disc: (0) absent [1,2,3,5,10,11,16]; (1) present**

[4,6,7,8,9,12,13,14,15]. The glands in the anterior and posterior attachment areas have been suggested to play a major role in attachment (Buchmann and Lindenstrom, 2002). This is a homoplastic character for the hypothesis. The presence of glands around the border of the oral disc, or state (1) (Figure 5), was shared by the macrovalvitrematids of the genera *Pseudotagia*, *Papillopseudotagia*, *Macrovalvitrematoides*, both species of the genus



*Macrovalvitrema*, *Pterinotrematoides* and *Neopterinotrematoides*. The hypothesis suggests the absence of glands as the plesiomorphic state followed by the change to state (1) in the common ancestor of all the macrovalvitrematids. For this character the hypothesis suggests a reversion of the character in the clade (Genus A, (*Nicolasia*, *Pseudohargisia*)) with the loss of glands around the oral disc. In addition, the presence of glands in the diclidophorid *Choricotyle* is suggested to have occurred independently.



**Figure 5.** Presence of glands around the oral disc of *Macrovalvitrema argentinensis* (Suriano, 1975). OS= oral suckers; Ph= pharynx; OD (dr)= Oral Disc (dorsal rim) and OD (vr)= Oral disc (ventral rim).

- 5. Position of male copulatory organ relative to intestinal bifurcation:** (0) anterior to intestinal bifurcation [14,15]; (1) at level of intestinal bifurcation [6]; (2) posterior to intestinal bifurcation [1,2,3,4,5,7,8,9,10,12,13,16]. The synapomorphy that represents the anterior position of the male copulatory organ in the species of *Pseudotagia* separates them from the rest of the ingroup (macrovalvitrematids). The position of this organ at level of the intestinal bifurcation, state (1) in *Macrovalvitrematoides micropogoni* as described by Hargis

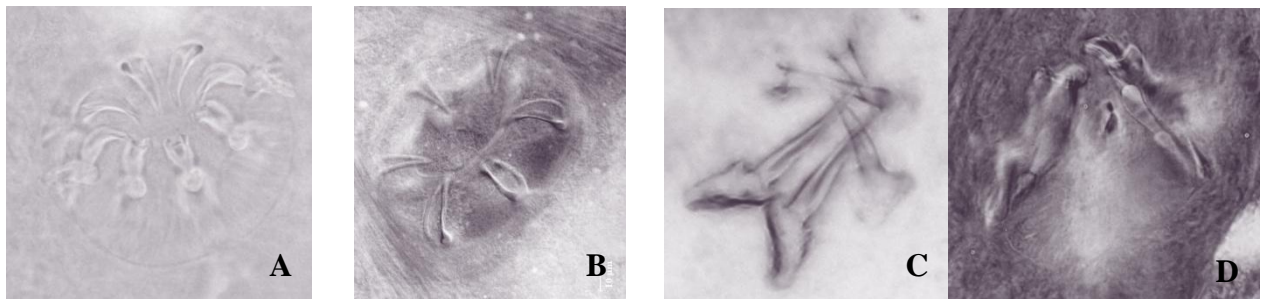
(1956) is an autapomorphy for the species, and is the only character that distinguishes this genus from *Macrovalvitrema*. The other described characters that separate these two genera were not included in the analysis because the type material of *M. micropogoni* was not available. State (2) which indicates the posterior position of the male copulatory organ is suggested by the hypothesis as the plesiomorphic state.

6. **Armature of male copulatory organ:** (0) unarmed [1]; (1) armed with spines arranged radially and closely [3,4,5,6,7,8,11,12,13,14,15] (Figure 6A); (2) armed with spines arranged radially and separated [10] (Figure 6B); (3) armed with spines arranged in different directions [2,9,16] (Figure 6C-D). The copulatory organ spines arranged radially and closely (State (0)) connected the members of the family Diclidophoridae with most of the members of the family Macrovalvitrematidae (Figure 6a). *Nicolasia canosorum* present state (2), radial arrangement of the spines, but widely separated (Figure 6B), which in the hypothesis is shown as an autapomorphy for this species. The hypothesis suggests that state (1) is the ancestral condition for all the macrovalvitrematids and the diclidophorids and is a homoplastic character because the ambiguity in the node that represents the most recent common ancestor for the clades (*Pterinotrematoides*, *Neopterinotrematoides*) and (Genus A, (*Nicolasia*, *Pseudohargisia*)).

The fast optimization (ACCTRAN) suggests a synapomorphy for the clade (*Pterinotrematoides*, *Neopterinotrematoides*) and (Genus A, (*Nicolasia*, *Pseudohargisia*)) and the reversion to state (0) in the clade (*Nicolasia*, *Pseudohargisia*).

The arrangement of the spines in different directions appears in *Pterinotrematoides mexicanum*, *Neopterinotrematoides avaginata* and Genus A. But the slow optimization (DELTRAN) suggests a synapomorphy for the clade that joins the diclidophorids and the macrovalvitrematids and is the independent transformation of the character in the clade (*Pterinotrematoides mexicanum*, *Neopterinotrematoides avaginata*) and Genus A.

The spines of *P. mexicanum* and *N. avaginata* have similar arrangement and shape of the spines having three pairs of different sizes arranged symmetrically, being the posterior pairs the largest and the most conspicuous (Figure 6C). Although Genus A, has its spines arranged in different directions, it has only five spines, with one larger central spine and two symmetrical pairs arranged bilaterally (Figure 6D). This could justify the independent development of this arrangement.



**Figure 6.** Armature of male copulatory organ. **A. State (1)** *Macrovalvitrema sinaloense* (Suriano, 1975), **B. State (2)** *Nicolasia canosorum* Suriano, 1975. **C State (3)** *Neopterinotrematoides avaginata* (Suriano, 1975) . **D State (3)** Genus A. . The first character state (0) is unarmed, meaning lacking distinguishable hard parts and is not included in this group of illustrations.

**7. Male copulatory spine shape: (0) none [1]; (1) all alike [2,3,4,5,6,7,8,11,12,13,14,15]**

(Figure 6A-B); **(2) all not alike [9,10,16]** (Figure 6C-D). The condition of all the spines different or not alike is only shown in the clade composed by *Pterinotrematoides* and *Neopterinotrematoides*. The fast optimization (ACCTRAN) suggests a synapomorphy for the

clade ((*Pterinotrematoides*, *Neopterinotrematoides*), (Genus A, (*Nicolasia*, *Pseudohargisia*))) and the posterior reversal of the character to the plesiomorphic condition in the clade composed by *Nicolasia* and *Pseudohargisia*). The slow optimization (DELTRAN) suggests independent transformation of the character in the clade (*Pterinotrematoides*, *Neopterinotrematoides*) and in Genus A.

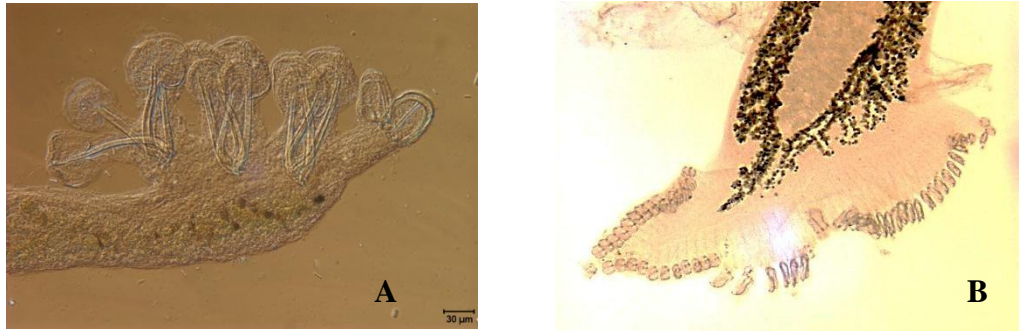
8. **Intestinal cecae end:** (0) not confluent [2,3,5,14,15]; (1) confluent [1,4,6,7,8,9,10,11,12,13,16]. This character is a symplesiomorphy for all the macroalvitrematid. Among the diclidophorids examined, only *Mamaevicotyle villalobosi* had the non-confluent state.
  
9. **Distribution of the intestinal cecae into the opisthaptor:** (0) not entering into the opisthaptor [1,2]; (1) one side branch entering [5]; (2) one branch extended to each peduncle [3,4]; (3) one dorsal branch entering [6,7,8,9,10,11,12,13,14,15,16]. There is an ambiguity among the ancestor of the diclidophorids and the difference in character state among the clade (*Eurysorchis*, *Choricotyle*) and *Mamaevicotyle*. Both the ACCTRAN and the DELTRAN suggest two synapomorphies, one for the clade (*Eurysorchis*, *Choricotyle*) having one intestinal branch extended to each peduncle, and one for the clade that composes the family Macroalvitrematidae having one dorsal branch.
  
10. **Distinction between body proper and opisthaptor:** (0) not constricted [2,3,5,6,7]; (1) constriction between body proper and the beginning of the opisthaptor [1,4,8,9,10,11,12,13,14,15]. This character establishes a homoplasy because state (1) occurs

independently in the clade (*Papillopseudotagia*, ((*Macrovalvitrematoides*, (*Macrovalvitrema sinaloense*, *M. argentinensis*)), ((*Pterinotrematoides*, *Neopterinotrematoides*), (Genus A, (*Nicolasia*, *Pseudohargisia*)))))) and in *Choricotyle* sp. and in *Pyragraphorus pyragraphorus*.

The hypothesis suggests that the only macrovalvitrematid genus that preserves the plesiomorphic state (0) is *Pseudotagia*. The information of this character for Genus A was not defined because the presence of a true constriction was not clearly observed. These worms are very narrow and elongate or stretched, and in most are folded at this level, but some seem to have a peduncle at the end of the body proper, anterior to the opisthaptor.

**11. Opisthaptor: (0) asymmetrical [1,2] (Figure 7A); (1) symmetrical**

**[3,4,5,6,7,8,9,10,11,12,13,14,15,16]** (Figure 7B). As a rule the attaching valves (clamps) are located symmetrically on the disc (opisthaptor) and the formation of an asymmetrical opisthaptor armed with valves (clamps) could originate in two ways (Bychowsky, 1957). In the first, apparently the more widespread, asymmetry occurs by a decrease in the number of valves (clamps) on one side of the opisthaptor, while the second type of asymmetry results in more or less equal number of valves on both sides of middle hooks (Bychowsky, 1957), but with differences in the clamp type or size providing the asymmetry. The asymmetrical state suggests synapomorphy for all the diclidophorids and all the macrovalvitrematids examined in this study. The other two outgroups develop different kinds of asymmetry, *A. hippos* has more clamps in one side than the other, while in *P. pyragraphorus* in addition to having more clamps on one side than the other also has two types of clamps with more or less the same number.



**Figure 7.** Symmetry of the opisthaptor. A. State (0), symmetrical opisthaptor as found in Genus A.; B. State (1) asymmetrical opisthaptor similar to those found in the outgroups *Allopyrgraphorus hippos* and *Pyragrphorus pyragrphorus*.

**12. Number of clamps:** (0) numerous [1,2]; (1) eight [3,4,5,6,7,8,9,10,11,12,13,14,15,16].

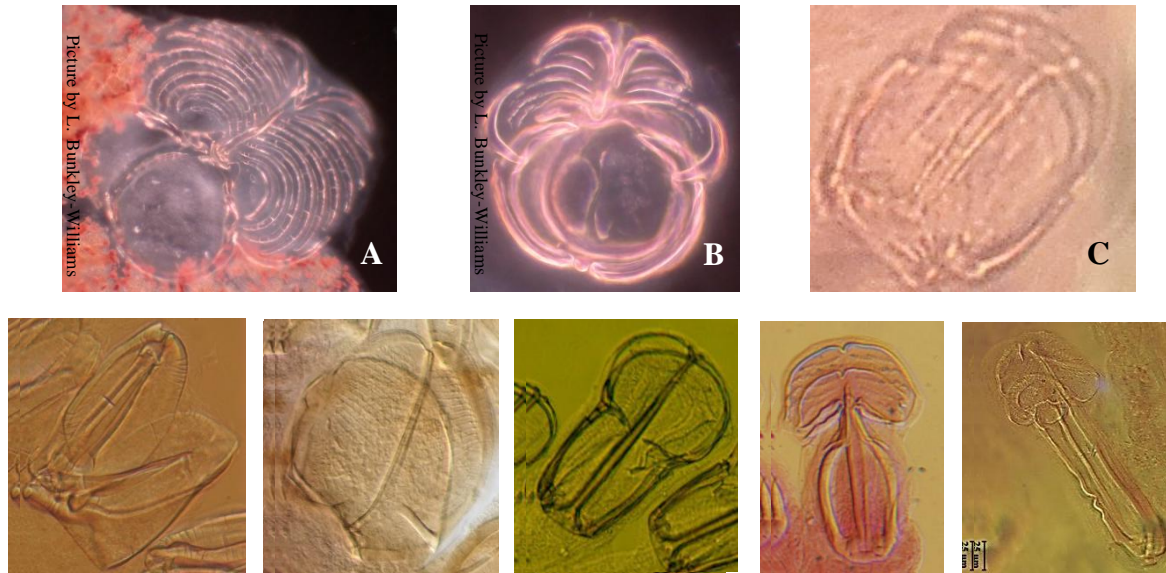
Having the character state (1), eight clamps corresponds to a synapomorphy for the diclidophorids with the macrovalvitrematids. *Allopyrgraphorus hippos* and *P. pyragrphorus* have numerous small clamps in their opisthaptor. According to the classification of Boeger and Kritsky (1993) among the superfamilies and families of the suborder Microcotylinea, the family Diclidophoridae (Superfamily Diclidophoroidea) is the only one that possesses eight clamps (four pairs).. The Macrovalvitrematidae also have an opisthaptor with eight clamps (four pairs), which suggests that these two families are the only two of the Suborder Microcotylinea with eight clamps. , For this reason Boeger and Kritsky (1993) included the macrovalvitrematids inside the Diclidophoridae since this would confer paraphyletic status to the diclidophorids..

**13. Clamp movement:** (0) short peduncles [1,5,14,15]; (1) long peduncles

[3,4,6,7,8,9,10,11,12,13,16]; (2) combined [2]. The macrovalvitrematids, except for members of the genus *Pseudotagia*, share state (1), presence of long peduncles. The hypothesis

suggests homoplasy in this character because of the independent acquisition of long peduncles by *Choricotyle*.

14. **Clamp style:** (0) diclidophorid round [3,4] (Figure 8A-B) ; (1) longer than wide (bivalved) [1,5,6,7,8,9,10,11,12,13,14,15,16] (Figure 8C-H); (2) wider than long and longer than wide (bivalved)[2]. The diclidophorid round clamps is proposed in the cladogram as a synapomorphy for the clade of diclidophorids (*Eurysorchis*, *Choricotyle*). All the macroalvitrematids together with the diclidophorid *Mamaevicotyle* have clamps longer than wide which correspond to the maintenance of the plesiomorphic state (1) or a symplesiomorphy. It is important to mention here, that among the subfamilies of Diclidophoridae the only one that does not possess the characteristic round clamps is the subfamily Diclidophorinae that includes *Mamaevicotyle villalobosi*. Although the clamps of members of this subfamily do not have the round shape, the sclerite composition and arrangement is similar to those in the other diclidophorid subfamilies (eg. *Eurysorchinae*, *Choricotylinae*), examined in this study.



**Figure 8.** Different clamp styles **A-C** Diclidophorid round style *Eurysorchis* sp., *Choricotyle* sp. and *Mamaevicotyle villalobosi* respectively. **D-H** Longer than wide style from macrovalvitremitids, **D.** *Pseudotagia*, **E.** *Papillopseudotagia*, **F.** *Macrovalvitrema*, **G.** *Nicolasia* and **H.** *Neopteriotrematoides*. The genera *Macrovalvitrematoides*, *Pseudohargisia* and *Pteriotrematoides* have similar clamp shape compared to *Macrovalvitrema*, *Nicolasia* and *Neopteriotrematoides* respectively.

**15. Relative number of clamps per shape: (0) all equal 1,3,4,5,14,15]; (1) 1:1 [2]; (2) 3:1**

sharing the same frame and sclerite arrangement (different in accessory sclerites) [6,7,8,13];

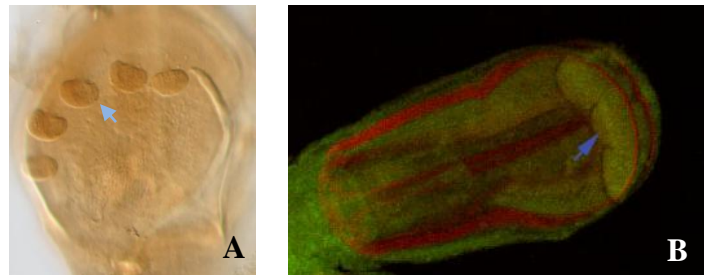
**(3) 3:1** not sharing the same frame and arrangement[9,10,11,12,16]. All the diclidophorids and the two species of the genus *Pseudotagia* maintained the plesiomorphic state (0) having eight equal clamps. It was noted in the genus *Pseudotagia* that one or two pairs depending on the species tended to be slightly smaller than the others but retained the same morphology.

The hypothesis suggests two synapomorphies, one for the rest of macrovalvitremitids which are in most inclusive position than *Pseudotagia* with the condition of three pairs of large clamps and one pair of small clamps that maintains the same frame and arrangement than the larger clamps. The genera *Papillopseudotagia*, *Macrovalvitrematoides* and *Macrovalvitrema* show the condition in which the small pair of clamps only differs from the larger by the



absence of an accessory structure that possibly augments the larger clamps' ability to produce suction. The three pairs of large clamps of *Papillopseudotagia* have five papillae at the border of the ventral valve (Figure 9A) that is absent in the small pair of clamps, while the three pairs of larger clamps of the genera *Macrovalvitrematoides* and *Macrovalvitrema* have a scalloped muscular pad at the border of the ventral valve (Figure 9B) that is also absent in the small pair of clamps.

Another synapomorphy is suggested for the clade ((*Pterinotrematoides*, *Neopterinotrematoides*), (Genus A, (*Nicolasia*, *Pseudohargisia*))) with a transformation to three pairs of large clamps and one pair of small clamps which have a different frame and sclerite arrangement than the large clamps and lack the characteristic strangulation present in the larger pair of clamps. The small pair of clamps of the genera *Nicolasia*, *Pseudohargisia* and Genus A is very similar in arrangement and shape, as well as those in the genera *Pterinotrematoides* and *Neopterinotrematoides* which are also very similar.

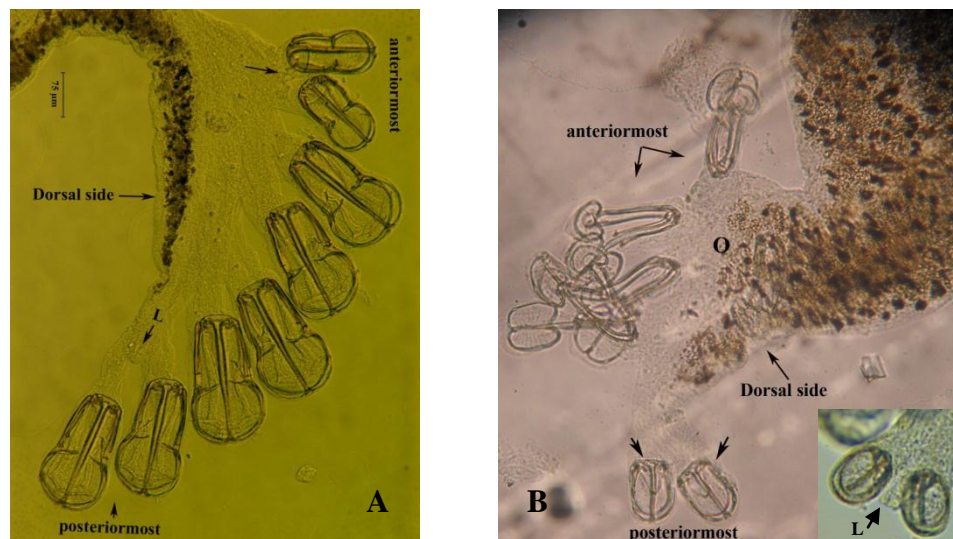


**Figure 9.** Accessory sclerites in the large clamps of *Papillopseudotagia hubbsi* (A) and the genera *Macrovalvitrematoides*, *Macrovalvitrema* and *Neomacrovalvitrema* (B). These structures are not present in the pair of small clamps of these taxa.

**16. Lappet:** (0) absent [1,2,3,4]; (1) present [5,6,7,8,9,10,11,13,14,15,16]. The presence of a lappet represents a synapomorphy for the macrovalvitrematids and the diclidophorid *Mamaevicole villalobosi*.

**17. Position of smaller clamps: (0)** anteriormost [6,7,8,13] (Figure 10a); **(2)** posteriormost [9,10,11,12,16] (Figure 10B). The hypothesis suggests a synapomorphy for the clade ((*Pterinotrematoides*, *Neopterinotrematoides*), (Genus A, (*Nicolasia*, *Pseudohargisia*))) having the pair of small clamps posterior to the larger pairs. The genera *Papillopseudotagia*, *Macrovalvitrematoides* and *Macrovalvitrema* is suggested to maintain the plesiomorphic state (0) having the pair of small clamps anterior to the larger pairs.

The genus *Pseudotagia* was not analyzed for this character because it does not have two kinds of clamps. However it is important to mention that the two species included in this analysis have slight difference in clamp size, where in *P. rubri* the posterior pair of clamps are smaller and in *Pseudotagia* sp. A both outer pairs are smaller than the central ones. This could be the ancestral conditions for the rest of macrovalvitrematids.



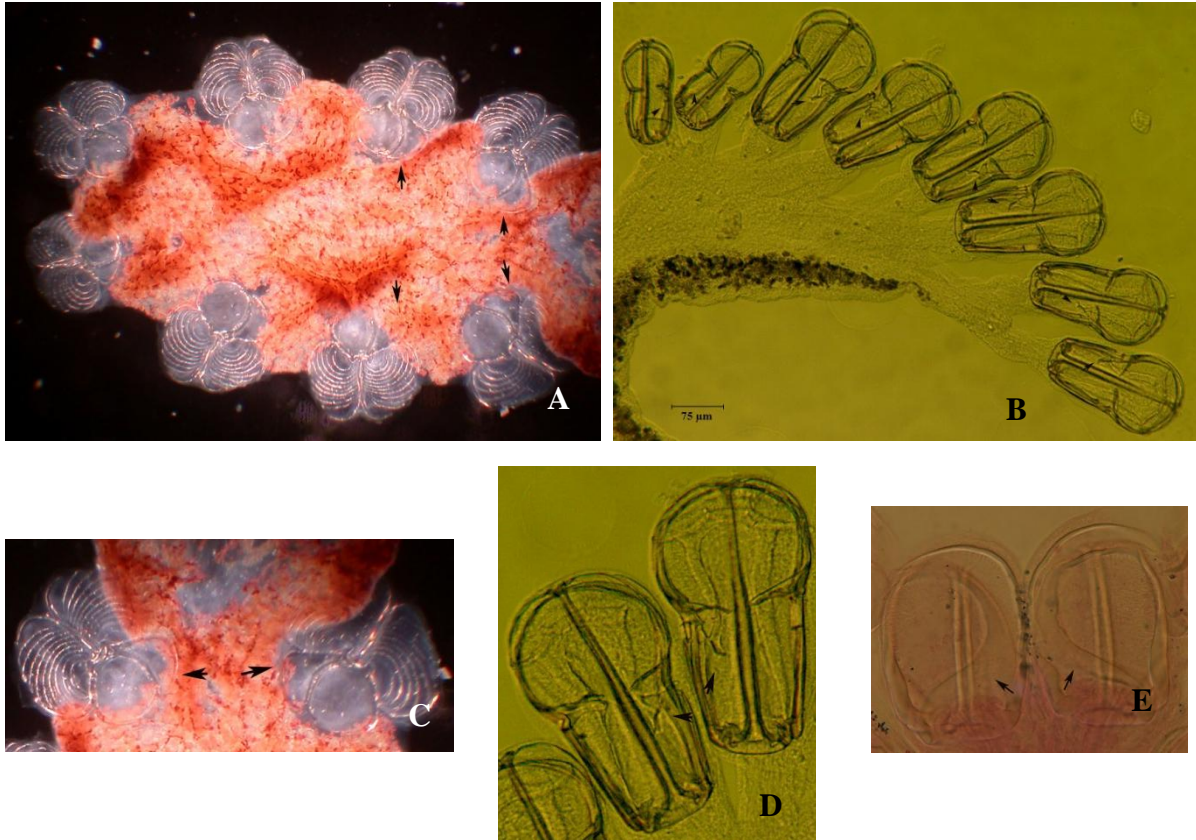
**Figure 10.** Position of the small pair of clamps. **A. State (0)**, Macrovalvitrematid (*Macrovalvitrema argentinensis*) with the small pair of clamps at anterior position on the opisthaptor which are the first pair of clamps and are oriented ventrally and widely distant from the lappet; **B. State (1)**, example of macrovalvitrematids with the small pair of clamps in a posteriormost position oriented posteriorly and with the lappet between and dorsal to their position (large photo *Neopterinotrematoides avaginata* and inset photo Genus A).

**18. Clamp symmetry: (0) symmetrical [1,2]; (1) asymmetrical**

[3,4,5,6,7,8,9,10,11,12,13,14,15,16] (Figure 11C-E). State (1), asymmetry of the clamps, is presented in the hypothesis as a synapomorphy for all the diclidophorids and macrovalvitrematids. For the majority of the diclidophorid genera the asymmetry of the right and left halves of the clamps as a result of the uneven development of the paired sclerites is characteristic (Bychowsky, 1957). Sproston (1946) considers the asymmetry of the sclerites of the clamps a mirror-like (bilaterally symmetrical, nobis) state on the right and left sides of the body as the basic characteristic peculiarity of Diclidophoridae (Bychowsky, 1957). The asymmetry of the clamps in both, the diclidophorids and the macrovalvitrematid monogenea examined occurs in the ventral loop or valve, but not in the dorsal loop or valve.

**19. Relative position of clamps: (0) no mirror image; (1) mirror image [3,4,5,7,8,9,10,13,16].**

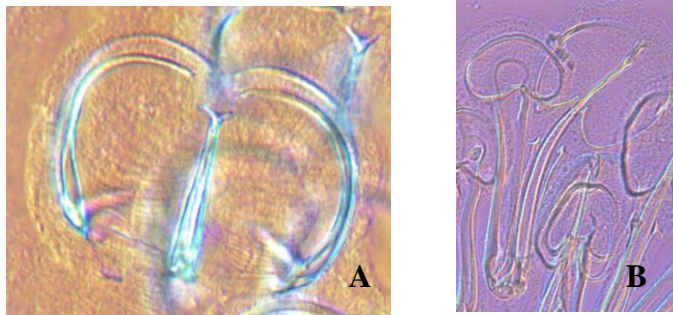
This character is uninformative because all the taxa with asymmetrical clamps examined in this study have a mirror image arrangement in the opisthaptor. This signifies that the clamps of each pair appeared or are positioned in the opisthaptor as mirror images of each other (Figure 11). In the macrovalvitrematids the clamps of each pair are positioned side by side, and the plates or their suture (fissure) of ventral valves of each clamp has a mirror image of the opposite clamp (Figure 11B). In the diclidophorids with round clamps, the arrangement is in pairs but are located on the outer edges of the opisthaptor and the mirror image is established by different sides of the ventral loop (valve) (Figure 11A).



**Figure 11** Mirror image arrangements of the asymmetrical clamps: **A-B.** Complete opisthaptor showing the mirror image of the four pairs of clamps in the diclidophorid *Eurysorchis* (**A**) and the macrovalvitrematid *Macrovalvitrema* (**B**). The second row (**C, D and E**) show the mirror image and the asymmetrical arrangement of: one pair of clamps of the diclidophorid *Eurysorchis* (**C**), one pair of large clamps of *Macrovalvitrema* (**D**), and one pair of the small of clamps of the macrovalvitrematid *Nicolasia* (**E**).

**20. Strangulation:** (0) without strangulation [1,3,4,5,13,14,15] (Figure 13A); (1) half of the clamps with and half without strangulation [2]; (2) slight strangulation (stays at lateral longitudinal level) [6,7,8] (Figure 13B); (3) pronounced strangulation at midline longitudinal level [9,10,11,12,16] (Figure 13C). The presence of strangulation of the lateral sclerites was observed in most of the macrovalvitrematids, except the genera *Pseudotagia* and *Papillopseudotagia*. Also the outgroup species *Pyragraphorus pyragraphorus* presents a strangulation of its lateral sclerites in one of its two kinds of clamps. But these clamps present a modification of their other shorter *Microcotyle* type clamps. The *Microcotyle*

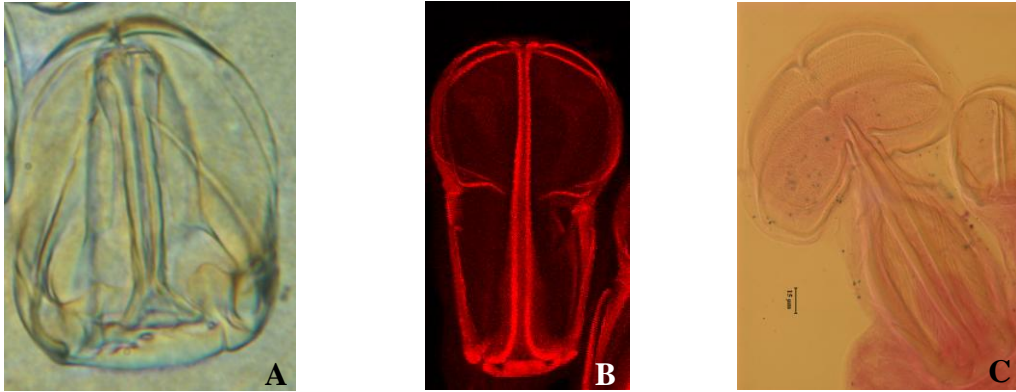
clamps are significantly different from the macrovalvitrematid clamps (Figure 12) where the lateral sclerites of both valves are equal. *Pyragraphorus pyragraphorus* presents the strangulation in the lateral sclerites of both valves. In the macrovalvitrematids the lateral sclerites of each valve are different and only the ones in the ventral valve present the strangulation.



**Figure 12.** Difference between a *Microcotyle* type clamp (A) and macrovalvitrematid clamp (B)

Among the macrovalvitrematids there are two kinds of strangulation of the lateral sclerites. One in which the lateral sclerites are slightly constricted toward the center of the clamps as occurs in the clade (*Macrovalvitrematoides micropogoni*, (*Macrovalvitrema sinaloense*, *M. argentinensis*)) (Figure 13B). The other has a narrowing of the lateral sclerites that occurs at the midline longitudinal level where the opposite lateral sclerites almost touch as occurs in the clade composed by ((*Pterinotrematoides*, *Neopterinotrematoides*), (Genus A, (*Nicolasia*, *Pseudohargisia*))) (Figure 13C).





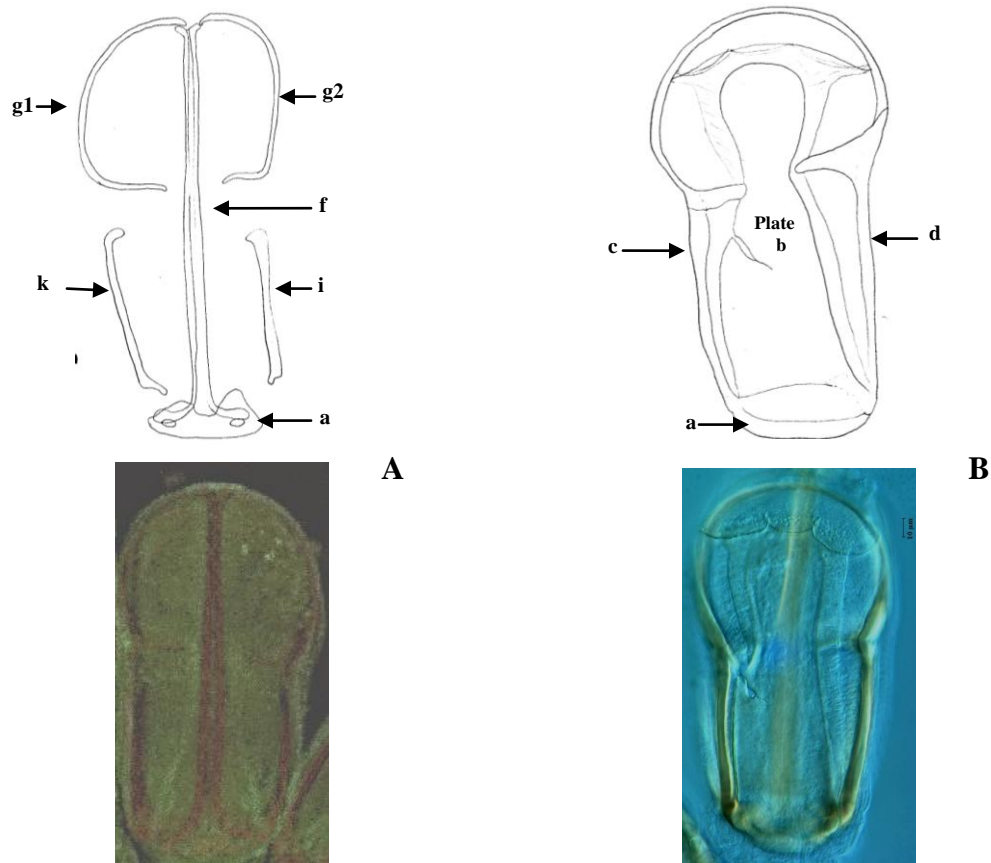
**Figure 13. States of strangulation of the lateral sclerites (character 20).** **A**,. State (0) *Pseudotagia*; **B** State (1) *Macrovalvitrema*; and **C** State (2) *Nicolasia*.

The hypothesis suggests two synapomorphies, one for each kind of strangulation, corresponding respectively to the clades (*Macrovalvitrematoides*, (*Macrovalvitrema sinaloense*, *M. argentinensis*)) and ((*Pterinotrematoides*, *Neopterinotrematoides*), (Genus A, (*Nicolasia*, *Pseudohargisia*))).

### **Clamp sclerite characters**

The characters discussed in this section include the information of the three pairs of large clamps and the single pair of small clamps of Macrovalvitrematidae separately because of the difference in their respective morphologies. The terminology used in the clamp description is based on the Caballero y C. and Bravo-Hollis (1955) descriptions. The labeling of the sclerites is made based on Rubec and Dronen (1993) for the clamps of the genus *Diclidophora*, which follows the Llewelyn (1958) scheme for labeling of sclerites. This includes the sclerites of the dorsal valve (g1 and g2, k and i; and f), referring to the distal lateral sclerites, proximal lateral sclerites and the median sclerite respectively; the ventral valve sclerites (c and d) referring to the ventral

lateral sclerites and the plate (b); and finally the (a) which was included by Rubec and Dronen (1993) as part of the ventral valve sclerites but in this study is referred to it as the basal sclerite.

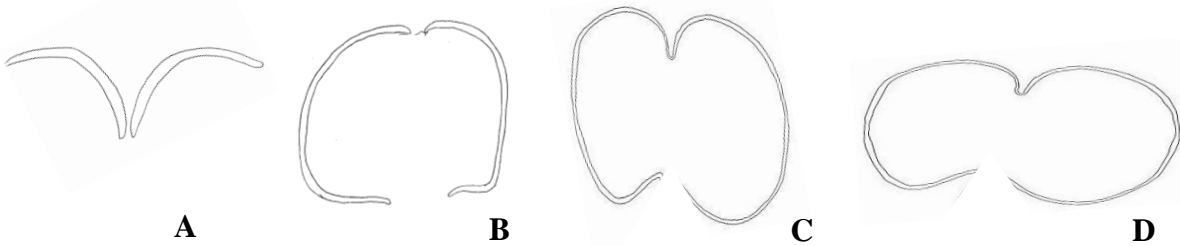


**Figure 14.** Identification of the clamp sclerites. These illustrations are of *Macrovalvitrema* Caballero y C. and Bravo-Hollis, 1955 the type genus of the family. **A.** Dorsal valve: **g1** and **g2**= distal lateral sclerites, **k** and **i**= proximal lateral sclerites, **f**, median sclerite, **a**= dorsal view of the basal sclerite; **B.** Ventral valve: **c** and **d**= lateral sclerites, **b**= plate and **a**= ventral view of basal sclerite.

## 21. Shape of g1 and g2 (distal segment of lateral sclerites of dorsal valve): (0) curved

[3,4,5,13] (Figure 15A); (1) semicircular [6,7,8] (Figure 15B); (2) butterfly-shaped [9,10,16] (Figure 15C); (3) laterally extended butterfly-shaped [11,12] (Figure 15D). The hypothesis suggests that state (0), curved g1 and g2 is the ancestral condition for the diclidophorids and macrovalvitrematids. This state is maintained and shared by the three species of diclidophorids examined and the macrovalvitrematid *Papillopseudotagia hubbsi*. The

hypothesis also established three synapomorphies; one for the clade (*Macrovalvitrematoides*, (*Macrovalvitrema sinaloense*, *M. argentinensis*)) having a semicircular shape in sclerites  $g_1$  and  $g_2$ , followed by the transformation of this character to a butterfly shape representing another synapomorphy for the clade ((*Pterinotrematoides*, *Neopterinotrematoides*), (Genus A, (*Nicolasia*, *Pseudohargisia*))) and another transformation in the most inclusive and apical clade (*Nicolasia*, *Pseudohargisia*) with a laterally extended butterfly shape in their sclerites  $g_1$  and  $g_2$ .

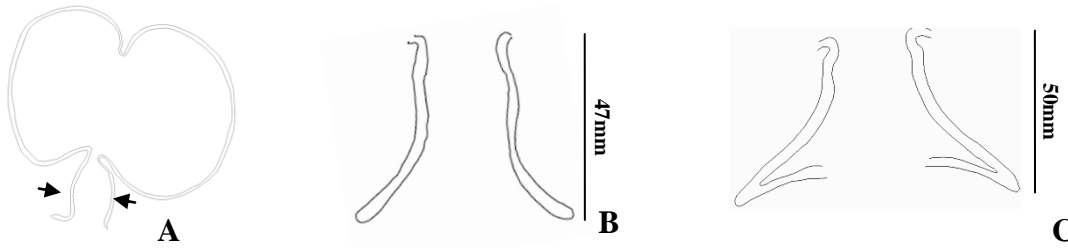


**Figure 15.** States of the shape of  $g_1$  and  $g_2$  (distal segment of lateral sclerites of dorsal valve). **A.** State (0) **curved**, as observed in the dieliodorphids, and in the macrovalvitrematid *Papillopseudotagia*; **B.** State (1), **semicircular**, found in the genera *Macrovalvitrema* and *Macrovalvitrematoides*; **C.** State (2), butterfly shaped,, seen in the genera *Pterinotrematoides*, *Neopterinotrematoides* and Genus A; **D.** State (3) laterally extended butterfly-shape, shared by the genera *Nicolasia* and *Pseudohargisia*.

## 22. Appendix in the $g_1$ and $g_2$ (distal lateral sclerites of the dorsal valve): (0) absent

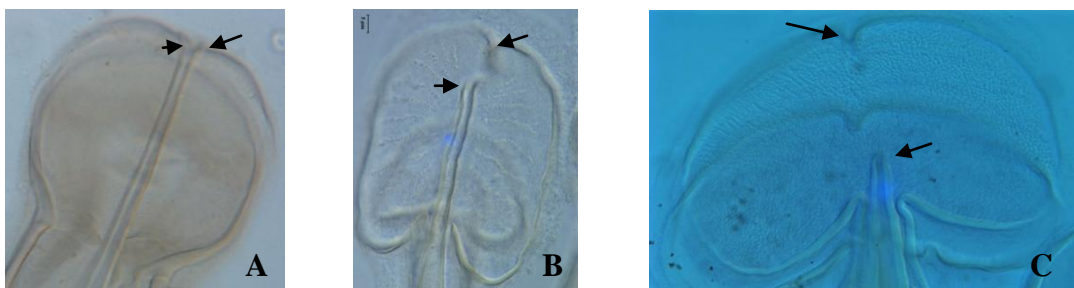
[3,4,5,6,7,8,9,10,13]; (1) hanging-sclerite-shaped [12,16] (Figure 16B); (2) wing-shaped [11] (Figure 16C). The presence of an appendix extending from a strangulation level is suggested as a synapomorphy for the clade (Genus A, (*Nicolasia*, *Pseudohargisia*)) and a posterior transformation of the character to a wing-shaped in *Nicolasia*.





**Figure 16.** Appendix of g1 and g2. **A.** Complete g1 and g2 showing appendix; **B. State (1)** Hanging sclerite, observed in the macrovalvitrematids *Pseudohargisia* and Genus A, and **C. State (2)** Wing-shaped appendix observed in *Nicolasia*.

**23. Extent of sclerite f (median sclerite of dorsal valve):** (0) superior to the distal end of g1 and g2 (articulates with them) [3,4,5,6,7,8,13] (Figure 17A); (1) close to g1 and g2 and but does not articulate with them [9,10,14,15] (Figure 17B); (2) distant from the distal end of the valve [11,12,16] (Figure 17C). All the diclidophorids and the macrovalvitrematid genera *Papillopseudotagia*, *Macrovalvitrematoides* and *Macrovalvitrema* share state (0) having the median sclerite articulating with sclerites g1 and g2, suggested in the hypothesis as the ancestral state for this character. The character state (1) represents a homoplasy because of having been acquired independently in the clade (*Pterinotrematoides*, *Neopterinotrematoides*) and the clade composed of the species of the genus *Pseudotagia*.



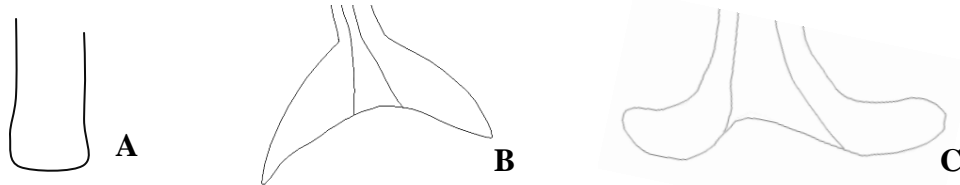
**Figure 17.** State of distal extent of median sclerite. **A.** State (0) (articulating with g1 and g2), *M. sinaloense*; **B.** State (1), close to g1 and g2 and but not articulating with it *N. avaginata*; **C.** State (2) distant from the distal end of the valve, *Nicolasia canosorum*.

The hypothesis suggests a synapomorphy for the clade (Genus, *Nicolasia*, *Pseudohargisia*) by having the distal end of the sclerite f (median sclerite) distant from the edges of the sclerites  $g_1$  and  $g_2$ .

**24. Distal end of f (median sclerite of dorsal valve):** (0) straight [3,4]; (1) slightly curved tips [9,10,11,12,16]; (2) curved tips [5,6,7,8,13,14,15]. All the macrovalvitrematids has some degree of curvature at the distal tip of sclerite f (median sclerite). The hypothesis suggested a synapomorphy for the clade composed of the diclidophorid *Mamaevicotyle* and all the macrovalvitrematids because of the presence of a curved distal tip of sclerite f (median sclerite). This condition is maintained in the macrovalvitrematid genera *Pseudotagia*, *Papillopseudotagia*, *Macrovalvitrematoides* and *Macrovalvitrema* and in the diclidophorid *Mamaevicotyle villalobosi*. Another synapomorphy occurs posteriorly in the most inclusive position with the transformation of the character to slightly curved tips in the clade ((*Pterinotrematoides*, *Neopterinotrematoides*), (Genus A, (*Nicolasia*, *Pseudohargisia*))).

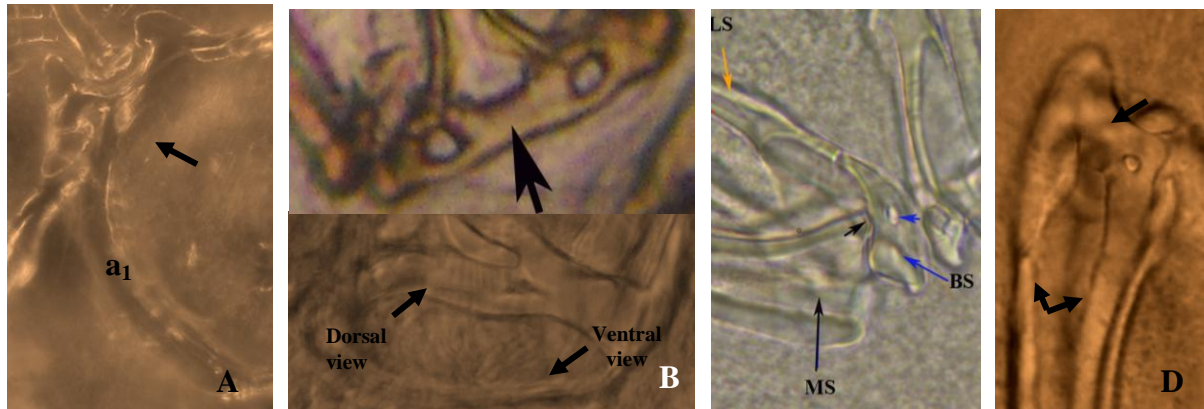
**25. Shape of basal end of sclerite f (median sclerite of dorsal valve):** (0) straight [3,4] (Figure 18A); (1) curved shape (pointed downward) [5,11,12,14,15,] (Figure 18B); (2) curved shape (pointed upward) [7,8,10,13,16] (Figure 18C). The hypothesis suggests a synapomorphy for the clade ((*Macrovalvitrematoides*, (*Macrovalvitrema*, *sinaloense*, *M. argentinensis*)), (*Papillopseudotagia*, ((*Pterinotrematoides*, *Neopterinotrematoides*), (Genus A, (*Nicolasia*, *Pseudohargisia*)))))) by having the end of the sclerite f (median sclerite) oriented toward the side. The posterior reversion of the character to a downward orientation of the basal end of

sclerite f (median sclerite) is also suggested in the most inclusive clade composed of *Nicolasia* and *Pseudohargisia* indicating an homoplasy.



**Figure 18.** States of basal end of sclerite f (median sclerite): **A.** State (0); **B.** State (1), and **C.** State (2).

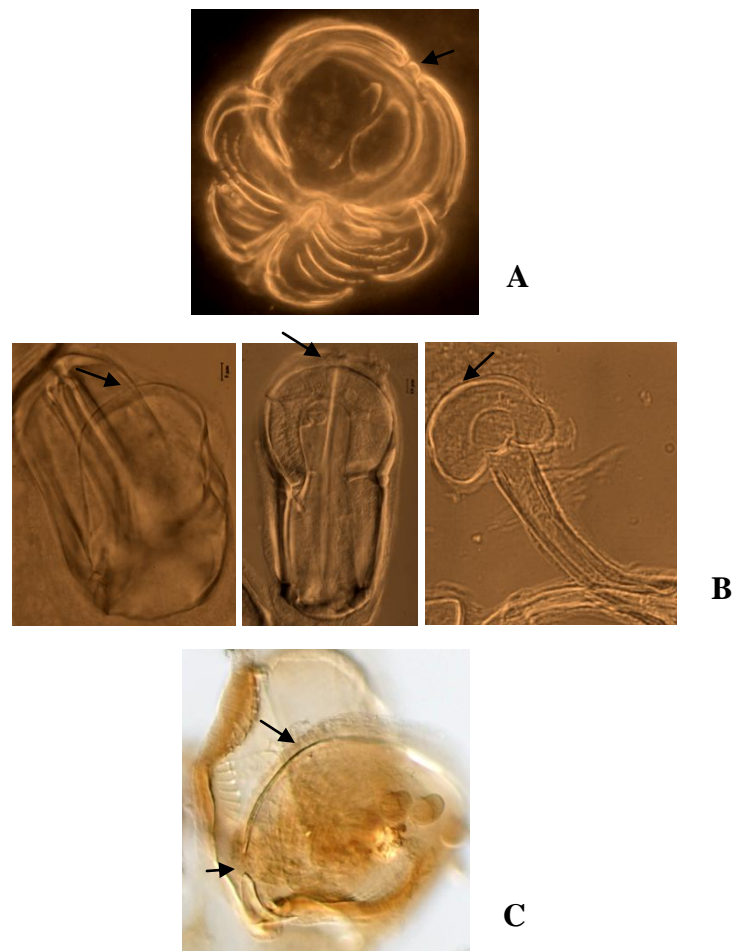
26. **Basal sclerite (a):** (0) pelvic ring-shaped with median sclerite ( $a_1$ ) [1,2], (Figure 19A); (1) pelvic ring-shaped without median sclerite [5,6,7,8,11,12,13,14,15] (Figure 19B); (2) open pelvic ring-shaped or U-shaped ( $90^\circ$ ) [9,10,] (Figure 19C); (3) U-shaped fused with the lateral sclerites c and d of the ventral valve [16] (Figure 19D). The hypothesis suggests the basal sclerite with a median sclerite ( $a_1$ ) as the plesiomorphic condition and is maintained in the diclidophorids with round clamps (*Eurysorchis*, *Choricotyle*). The hypothesis suggests that the clade (*Pterinotrematoides* and *Neopterinotrematoides*) shows a synapomorphy by having U-shaped pelvic ring basal sclerite, and the autapomorphy for Genus A because it has the U-shaped basal sclerite fused with the lateral sclerites of the ventral valve.



**Figure 19.** States of the sclerite a (basal sclerite). **A.** State (0), characteristics of diclidophorids,  $a_1$  = median sclerite of basal sclerite; **B.** State (1), as found in most of the macrovalvitrematids; **C** State (2) found in the macrovalvitrematids genera *Pterinotrematoides* and *Neopterinotrematoides*, this is a side view in which the articulation of the basal sclerite with the median sclerite (MS) is observed; **D.** State (3) found as an autapomorphy in Genus A, the picture is a ventral view of the basal sclerite showing it fused with the lateral sclerites c and d of the ventral valve.

## 27. Distal end of sclerites c and d (lateral sclerite of ventral valve): (0) not fused distally

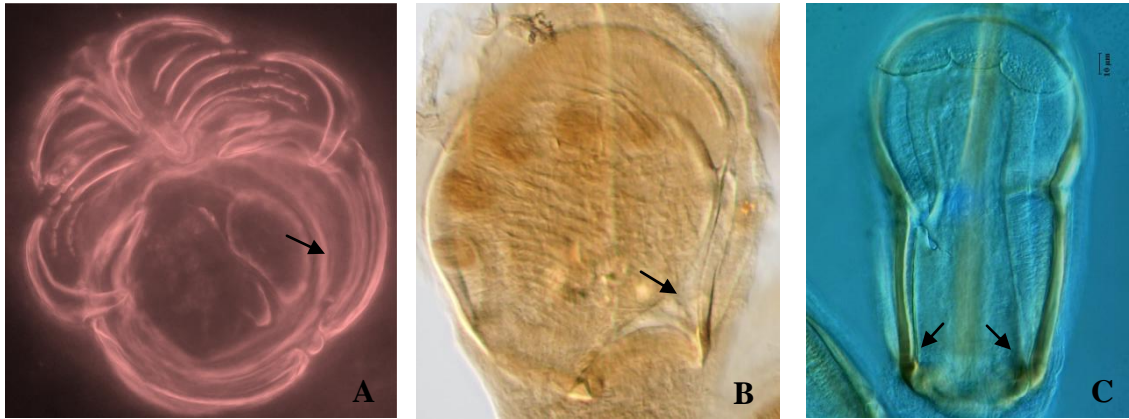
[1,2,3,4,5] (Figure 20A); (1) fused distally [6,7,8,9,10,11,12,14,15,16] (Figure 20B); (2) fused distally, not fused at one lateral side [13] (Figure 20C). State (0) is plesiomorphic for all the diclidophorids and macrovalvitrematids and is maintained as a symplesiomorphy for the diclidophorids. This character appears as a synapomorphy for all the macrovalvitrematids by having the distal end of the sclerites c and d (lateral sclerites of ventral valve) fused and having the distal end of these sclerites fused distally but opens at side of the sclerite c is suggested an autapomorphy for *Papillopseudotagia*.



**Figure 20.** States of the sclerites c and d (lateral sclerites of ventral valve). **A.** State (0) the sclerites c and d are not fused distally; **B** State (1). found in some macrovalvitrematid taxa with sclerites c and d fused distally; **C.** State (2) found in *Papillopseudotagia* sclerites c and d, are fused distally but open or separated in one lateral side.

**28. Articulation of plate of ventral valve (b):** (0) absent [1,2]; (1) reduced to the side of the sclerite c [4,5] (Figure 21A-B); (2) occupy the proximal area from lateral sclerite c to lateral sclerite d [6,7,8,9,10,11,12,13, 14, 15,16] (Figure 21C). This plate was not observed in the diclidophorid *Eurysorchis*, but the other two diclidophorids examined were found to have this plate reduced and limited to the side of sclerite c (1). The hypothesis suggests the synapomorphy for all the macrovalvitrematids with the plate occupying the proximal area

extending from sclerite c to sclerite d and the posterior reversion of the character to a plate reduced along sclerite c in *Papillopseudotagia*.



**Figure 21.** States of the proximal position of the plate (b) of the ventral valve. The arrows indicate the position of the plate. **A.** State (1) the clamp of the diclidophorid *Choricotyle* with the plate limited to the side enclosed by sclerite c of the ventral loop (valve) ); **B** Also state (1) the clamp of *Papillopseudotagia hubbsi* with the plate limited to the side enclosed by sclerite c; and **C.** State (2) the clamp of *Macrovalvitrema* with the plate covering the area from the side of lateral sclerite c to the side of the lateral sclerite d,

**29. Vertical extent of the plate (b): (0)** extended only to proximal part of the ventral valve

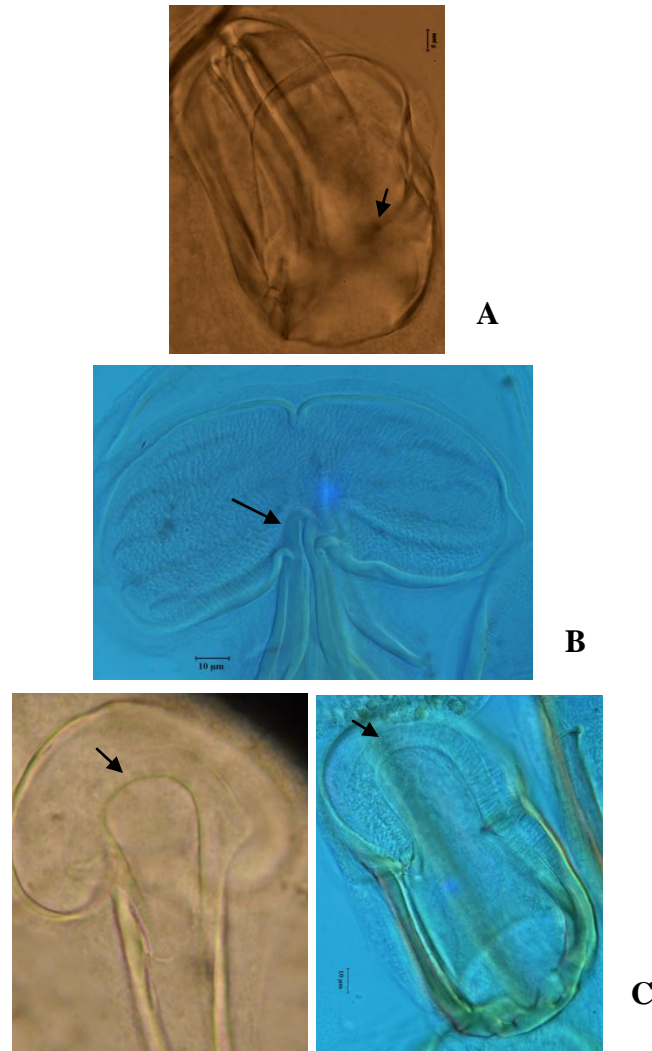
[14,15] (Figure 22A); **(1)** extended to level of strangulation [9,11,12,16] (Figure 22B); **(2)**

extended to distal segment [6,7,8,10] Figure 22C). The vertical extent of the plate in

*Papillopseudotagia* and the diclidophorids *Choricotyle* and *Mamaevicotyle* were difficult to determine.

In the clade composed of the species of *Pseudotagia*, this plate is limited to the proximal extent of the ventral valve (state 0), suggested by the hypothesis as the plesiomorphic condition of this character. The fast optimization (ACCTRAN) suggests synapomorphy for all the macrovalvitrematids except members of the genus *Pseudotagia*, which are in the most exclusive position. It also indicates the independent transformation of the character to a plate

extended to the distal level as homoplasy for the clade (*Macrovalvitrematoides*, (*Macrovalvitrema sinaloense*, *M. argentinensis*)) and *Neopterintrematoides*. The slow optimization suggests the synapomorphy occurring in most inclusive position in the clade ((*Pterintrematoides*, *Neopterintrematoides*), (Genus A, (*Nicolasia*, *Pseudohargisia*))))



**Figure 22.** Vertical extent of the plate (b) of the ventral valve. **A.** State (0) observed in species of *Pseudotagia*, photo is of *Pseudotagia* sp. A.; **B.** State (1) observed in genera *Pterintrematoides*, Genus A., *Nicolasia* and *Pseudohargisia*, photo is of *Nicolasia* (Suriano, 1975). **C.** State (2) the plate extends to the distal segment and is bordered by a thick wall of muscle. Photos of *Neopterintrematoides avaginata* (left) and *Macrovalvitrema argentinensis* (right).

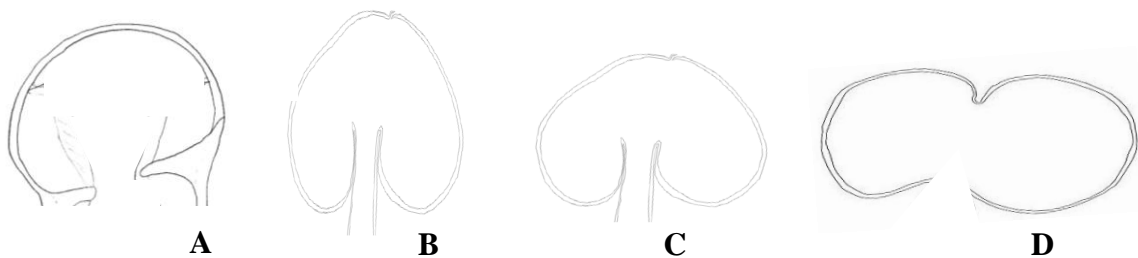


**30. Presence of diaphragm (sucker pad) in ventral loop or valve: (0) absent**

[1,2,5,6,7,8,9,10,11,12,13,14,15 16]; (1) present [3,4]. This character represents a synapomorphy with the presence of a diaphragm only in the clade of diclidophorids with round clamps (*Eurysorchis*, *Choricotyle*).

**31. Shape of the distal part of sclerites c and d (lateral sclerites of the ventral valve)\* this**

character only applies to those clamps with a strangulation: (0) semicircular [6,7,8] (Figure 23A); (1) oval-shaped [9] (Figure 23B); (2) cardioid-shaped [10,16] (Figure 23C) (3) laterally extended butterfly-shaped [11,12] (Figure 23D).



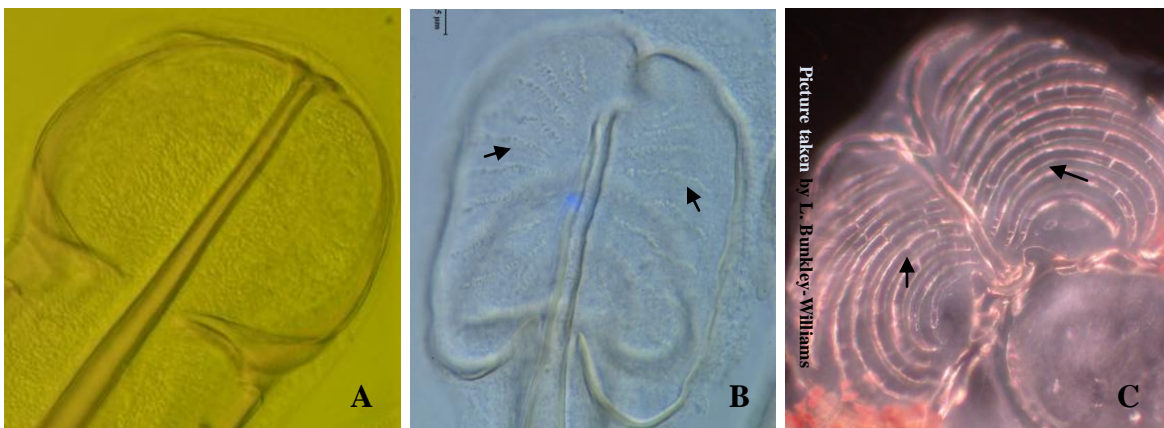
**Figure 23.** Shape of the distal part of sclerites c and d (lateral sclerites of the ventral valve): **A.** State (0) found in the genera *Macrovalvitrema* and *Macrovalvitrematoides*; **B.** State (1) which corresponds to the autopomorphy found in *Pterinotrematoides mexicanum*; **C.** State (2) the cardioid-shaped found in the genera *Neopterinotrematoides* and Genus A; and **D.** State (3) extended butterfly-shaped that occurs as a synapomorphy for the genera *Nicolasia* and *Pseudohargisia*.

The clade (*Macrovalvitrematoides*, (*Macrovalvitrema sinaloense*, *M. argentinensis*)) share a semicircular shape, but the hypothesis does not specify if this constitutes a synapomorphy or if it really is a symplesiomorphy. The hypothesis suggests synapomorphy for the clade (*Nicolasia* and *Pseudohargisia*). The ACCTRAN and DELTRAN present two competing hypotheses about where the node (common ancestor) begins the transformation of the character that establishes the synapomorphy for the condition of a cardioid shape in *Neopterinotrematoides* and Genus A. For this character the hypothesis also suggests an



autapomorphy in *Pterinotrematoides mexicanum* by having the sclerites c and d with an oval shape.

**32. Ornamentation in the dorsal loop or valve:** (0) absent [1,2,5,6,7,8,14,15] (Figure 24A); (1) bars [9,10,11,12,13,16] (Figure 24B); (2) rib-like ornamentation [3,4] (Figure 24C).



**Figure 24.** Ornamentation in the distal segment of dorsal valve (or loop). **A.** State (0), includes those taxa without any ornamentation as in the genera *Macrovalvitrematoides* and *Macrovalvitrema* and *Pseudotagia*; **B.** State (1), includes those genera with bars as genera *Pterinotrematoides*, *Neopterinotrematoides*, *Nicolasia*, *Pseudohargisia*, *Papillopseudotagia*, and Genus A; and **C.** State (2) includes those with ribs as the two diclidophorid with round clamps included in the study (*Eurysorchis* and *Choricotyle*).

The hypothesis suggests two synapomorphies, one for the clade (*Eurysorchis*, *Choricotyle*) with the character state (2) which implies the presence of ribs and one for the clade (*Papillopseudotagia*, ((*Pterinotrematoides*, *Neopterinotrematoides*), (Genus A, (*Nicolasia*, *Pseudohargisia*)))) with character state (1) which implies the presence of bars. The rest of the macrovalvitrematids including the genera *Pseudotagia*, *Macrovalvitrematoides* and *Macrovalvitrema* together with the diclidophorid *Mamaevicotyle* maintain a symplesiomorphy, by having the ancestral condition represented by state (0) or the absence of ornamentation.

**33. Ornamentation in the ventral loop or valve: (0) absent [1,2,3,4,5,6,7,8,9,10,14,15,16]**

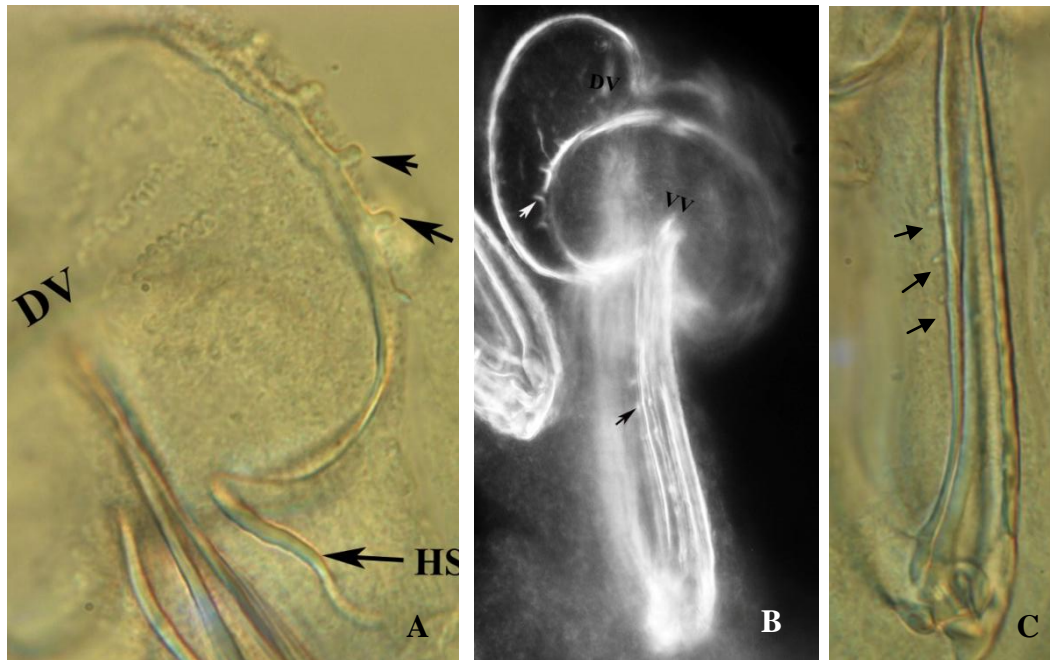
(Figure 25A); (1) bars [11,12,13] (Figure 25B-C). According to the hypothesis this is a homoplastic character establishing the development of bars or state (1) independently for the clade (*Nicolasia*, *Pseudohargisia*) and in *Papillopseudotagia*. The rest of the macrovalvitrematids as well the diclidophorid do not present any ornamentation on this valve retaining what is suggested in this hypothesis as the plesiomorphic state (0) for this character.



**Figure 25.** Presence of ornamentation on the ventral valve: **A.** State (0) ventral valve without ornamentation as in most of the macrovalvitrematids (*Macrovalvitrematoides*, *Macrovalvitrema*, *Pterinotrematoides*, *Neopterinotrematoides*, *Pseudotagia* and Genus A); **B-C** State (1) presence of bars along the ventral valve, the pictures C and D are referring to the bars observed in *Papillopseudotagia*, and *Nicolasia* and/or *Pseudotagia*.

**34. Lateral ornamentation: (0) absent [1,2,3,4,5,6,7,8,9,10,11,12,13,14,15]; (1) present [16].**

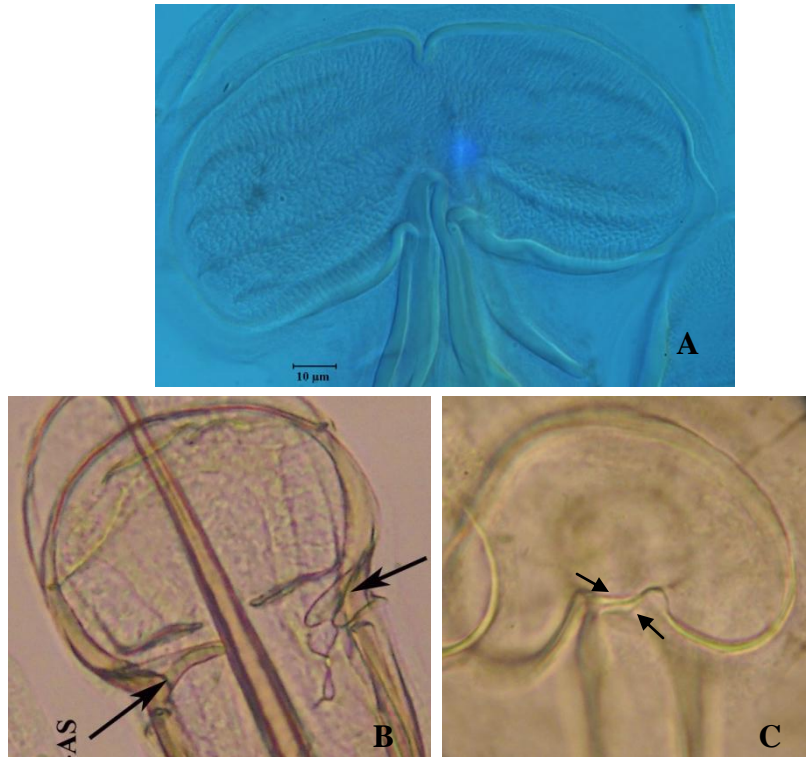
This character corresponds to an autopomorphy for Genus A. This was included in the analysis to distinguish Genus A from the rest of the macrovalvitrematids. The lateral sclerites of both valves including ( $g_1$  and  $g_2$ ) of the dorsal valve and both distal and proximal part (c and d) of the ventral valve of this species are armed with short and flat spines. The sclerites  $g_1$  and  $g_2$  have seven of these short and flat spines, while the sclerites c and d have five in the distal part and three in the proximal and slender part (Figure 26).



**Figure 26.** Lateral ornamentation: All photos of Genus A, which has state (1) for this character as an autapomorphy. **A.** Short and flat spines in the lateral side of  $g_1$  or  $g_2$  of the dorsal valve; **B-C** Spines in sclerite c or d of the ventral valve, C three spines in the proximal part of the lateral sclerite c or d.

**35. Accessory sclerites at strangulation:** (0) absent [9,11,12,16] (Figure 27A); (1) asymmetric accessory sclerites [6,7,8] (Figure 27B); (2) symmetric accessory sclerites [10] (Figure 27C).

Among the macrovalvitrematids with a strangulation of the lateral sclerites there are three conditions or states. The presence of asymmetric accessory sclerites at the level of the strangulation is suggested by the hypothesis as a synapomorphy for the clade (*Macrovalvitrematoides*, (*Macrovalvitrema sinaloense*, *M. argentinensis*)). Also the maintenance of the plesiomorphic state is suggested in the other macrovalvitrematids with strangulation except for *Neopteriotrematoides* that changed from the ancestral condition (state 0) to state (2) acquiring one pair of symmetrical accessory sclerites at the strangulation level suggested as an autapomorphy.



**Figure 27.** Accessory sclerites at the strangulation level: **A.** State (0); **B.** State (1), which comprises a synapomorphy for the genera *Macrovalvitrematoides* and *Macrovalvitrema*; and **C.** State (2) which is an autapomorphy for the genus *Neopterinotrematoides*.

**36. Serration of lateral sclerites:** (0) absent [2,3,4,5,6,7,8,9,10,11,12,13,15,16]; (1) present in ventral valve [1]; (2) present in both valves [14]. In the hypothesis that resulted from this analysis, the presence of serration represents an autapomorphy for *Pseudotagia rubri*, but this character was maintained in the analysis for two reasons: First, although the species *P. cupida* was not included in the study, Hargis (1956) described these valves as serrated. This detail could be considered as a synapomorphy for *P. rubri* and for *P. cupida*. Second, the new species of *Pseudotagia* proposed in this study does not have serrations in its clamps; and third, this character can be used in the future to distinguish the other species of *Pseudotagia*.

### Characters of the small pair of clamps

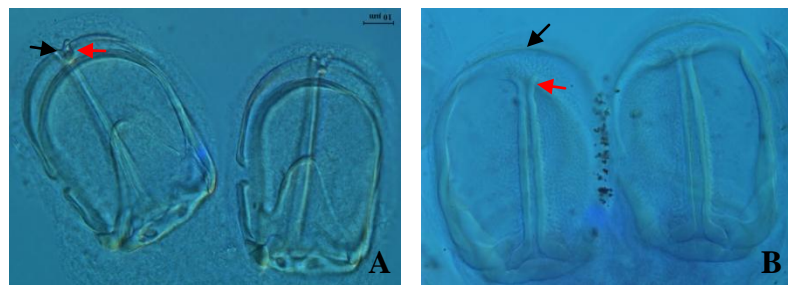
Except for the genus *Pseudotagia*, the macrovalvitrematids have three pair of large and one pair of small clamps. The small pair in some species is only slightly different from the large pair, while in the others it is significantly distinctive from the larger pairs. The ones that are slightly different from the three larger pairs are differentiated by the absence of accessory sclerites (see discussion Character 15, Figure 9A-B). The pair of small clamps can be located either anterior of the large clamps or posterior to them. Those in the anterior position related the genera *Macrovalvitrematoides*, *Macrovalvitrema* and *Papillopseudotagia*; and those in the posterior position related the macrovalvitrematid genera *Pterinotrematoides*, *Neopterinotrematoides*, *Nicolasia*, *Pseudohargisia* and Genus A. This will be discussed in the sections describing characters selected for the small pairs of clamps in the genera *Macrovalvitrematoides*, *Macrovalvitrema*, *Pterinotrematoides*, *Neopterinotrematoides*, *Nicolasia*, *Pseudohargisia*, *Papillopseudotagia* and the Genus A.

**37. Presence of strangulation (in small pair of clamps):** (0) absent [9,10,11,12,13,16] (Figure 28A); (1) present [6,7,8] (Figure 28B). The hypothesis suggests that the presence of strangulation is a synapomorphy for the clade (*Macrovalvitrematoides*, (*Macrovalvitrema sinaloense*, *M. argentinensis*)). The other macrovalvitrematids maintains the plesiomorphic state (0).





**Figure 28.** States of strangulation in the small pairs of clamps. **A.** State (0) and **B** State (1) which according to the hypothesis suggests a synapomorphy for the clade of genera *Macrovalvitrematoides* and *Macrovalvitrema*.



**Figure 29.** Sclerites of the dorsal valve of the small pair of clamps **A.** Sclerites  $g_1$  and  $g_2$  not fused distally and median sclerite (f) exceeding the edge of  $g_1$  and  $g_2$  (red arrows). **B.** Sclerites  $g_1$  and  $g_2$  fused distally and median sclerite not reaching the edge of the sclerites  $g_1$  and  $g_2$ .

### 38. Distal end of sclerites $g_1$ and $g_2$ (lateral sclerites of dorsal valve) in small pair of clamps:

(0) not fused distally [7,8,9,10,13] (Figure 29A); (1) fused distally [11,12,16] (Figure 29B).

Having the sclerites  $g_1$  and  $g_2$  distally fused is suggested by the hypothesis as a synapomorphy for the clade (Genus A, (*Nicolasia*, *Pseudohargisia*)).

### Comparison with the pairs of large clamps

This state is exclusive for the small pair of clamps of these genera, because the large pairs maintain the plesiomorphic state (0) that refers to the sclerites  $g_1$  and  $g_2$  not fused distally.

The genera *Macrovalvitrema*, *Pterinotrematoides*, *Neopterinotrematoides* and *Papillopseudotagia* maintain the plesiomorphic state (0).

**39. Extention of sclerite f (median sclerite) of the dorsal valve of pair of small clamps: (0)**

not reaching the edge of sclerites  $g_1$  and  $g_2$  [11,12,16] (Figure 29B); (1) exceeding the edge of sclerites  $g_1$  and  $g_2$  [7,8,9,10,13] (Figure 29A). The hypothesis suggests a synapomorphy for the clade (Genus A, (*Nicolasia*, *Pseudohargisia*)) with the sclerite f (median sclerite) distant from the edge of  $g_1$  and  $g_2$ .

**Comparison with the pairs of large clamps**

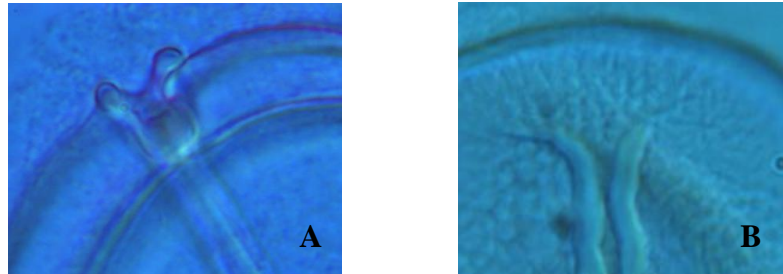
The large clamps of these genera (Genus A, *Nicolasia* and *Pseudohargisia*) also have the distal end of the sclerite f (median sclerite) not reaching the edge of  $g_1$  and  $g_2$ .

The genera *Papillopseudotagia*, *Macrovalvitrema*, *Pterinotrematoides* and *Neopterinotrematoides* maintain their sclerite f articulating or exceeding the edge of the sclerites  $g_1$  and  $g_2$ , which suggests the plesiomorphic state for the macrovalvitrematids. Contrary to this apparent symplesiomorphy, the sclerite f (median sclerite) of the three pairs of large clamps of the genera *Pterinotrematoides* and *Neopterinotrematoides* does not reach or articulate with the edges of  $g_1$  and  $g_2$ , separating them from *Papillopseudotagia*, *Macrovalvitrematoides* and *Macrovalvitrema*).

**40. Shape of distal end of sclerite f (median sclerite) of small clamps: (0) slightly curved**

[11,12,16]; (1) distinctively curved [7,8,9,10, 13]. These character suggests the same relations as the previous two characters (distal end of  $g_1$  and  $g_2$  and extention of the sclerite f)

establishing a synapomorphy for the clade (Genus A, (*Nicolasia*, *Pseudohargisia*)), and the apparent symplesiomorphy or maintenance of the plesiomorphic state (1) in the others.



**Figure 30. States of the distal end of the sclerite f (median sclerite) of small clamps: A. State (0); B. State (1).**

#### **Comparison with the pairs of larger clamps**

The large pairs of clamps of genera Genus A, *Nicolasia* and *Pseudohargisia* also have this state (slightly curved distal end) in the sclerite f. But this character in the large clamps is also shared (synapomorphy) with the genera *Pterinotrematoides* and *Neopterinotrematoides*.

- 41. Shape of basal end of sclerite f (median sclerite) in pair of small clamps: (0) curved downward [11,12,13] (Figure 30 A); (1) curve upward [7,8,9,10,16] (Figure 30 B).** The hypothesis suggests that the state (1) establishes a synapomorphy for the clade (*Nicolasia*, *Pseudohargisia*).

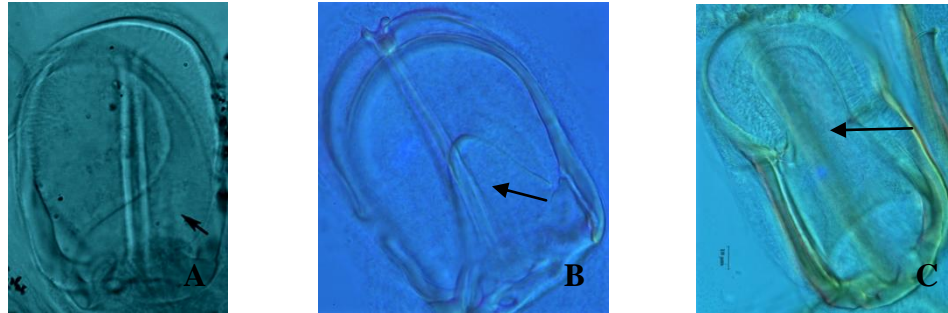
#### **Comparison with the pairs of large clamps**

This clade is also supported by this state in the pairs of large clamps (Character 25, Figure 18B).



**42. Extent of the plate (b) in the pair of small clamps: (0) oriented toward sclerite c**

[11,12,13,16] (Figure 31A); (1) medially positioned reaching the median level of the ventral valve [9,10] (Figure 31B; (2) medially positioned extended along whole valve [6,7,8] (Figure 31C).



**Figure 31.** States of the extent of the plate (b) in pair of small clamps. **A.** State (0) found in genera *Nicolasia*, *Pseudohargisia*, *Papillopseudotagia* and Genus A; **B.** State (1) observed in genera *Pterinotrematoides* and *Neopterinotrematoides*; and **C.** State (2) found in the genera *Macrovalvtirematoides* and *Macrovalvitrema*.

The hypothesis suggests that the plesiomorphic state is the plate oriented toward sclerite c of the ventral valve, and this state is found in the genus *Papillopseudotagia* and in the clade (Genus A, (*Nicolasia*, *Pseudohargisia*)). Two synapomorphies are also suggested, one for the clade (*Macrovalvitrematoides*, (*Macrovalvitrema sinaloense*, *M. argentinensis*)) with the plate medially positioned and extended along complete valve and one for the clade (*Pterinotrematoides*, *Neopterinotrematoides*) having the plate medially positioned at median level of the clamp.

**Comparison with the pairs of large clamps**

**First:** The three pairs of large clamps of *Macrovalvitrematoides* and *Macrovalvitrema* maintains the same extent of the plate as the small pair.

**Second:** *Pterinotrematoides* maintains a similar extent, but in the larger clamps the plate reaches the strangulation level.

**Third:** The pairs of large clamps of *Neopterinotrematoides* do not maintain the same arrangement as their small clamps. They are more related to the genera *Macrovalvitrematoides* and *Macrovalvitrema* by having the plate extending to the distal segment of the valve, which attributed a homoplasy to the hypothesis (see Character 29, Figure 22C). Contrary to this, their pair of small clamps maintains a synapomorphy with the pairs of small clamps of *Pterinotrematoides* by having the plate medially positioned extending until the median level of the valve (Figure 31B).

**Fourth:** The pairs of large clamps of *Papillopseudotagia* is the only macrovalvitrematid that shows the plate positioned along sclerite c of the ventral valve (1), the character state also seen in the small pair of clamps (see Character 28, Figure 21). This is suggested by the hypothesis as a homoplasy being considered a reversion of the character to the condition presented by the diclidophorids.

Character state (0) in the small pair of clamps is considered by the hypothesis as plesiomorphic and is also maintained in the most inclusive clade of the hypothesis (Genus A, (*Nicolasia*, *Pseudohargisia*)). In conclusion in both the large and small clamps, the condition of having a reduced plate oriented beside sclerite c represents an ancestral condition.

### **Characters not included:**

Number and position of vaginal openings:

The vagina in monogeneans is reported to present numerous positions in the body as well as variable number of vaginae and vaginal pores. It can be positioned dorsally, ventrally and laterally and the numbers can vary between one and two and with one, two or numerous vaginal pores. According to Bychowsky (1957) the vaginal duct can be present or absent, and in a majority of cases it is a single duct starting from the oviduct or two vaginal ducts emerging from the vitelline ducts. These vaginal ducts are variously located among different species Bychowsky (1957).

Boeger and Kritsky (1993) postulated the transformation of states of vaginae in the monogeneans establishing several states for this character: one midventral “true” vagina, one ventrolateral “true vagina”, two lateral “true” vaginae, vagina absent, two lateral “ductus vaginalis”, one ventrolateral “ductus vaginalis”, one midventral “ductus vaginalis”, one middorsal “ductus vaginalis” and two dorsal “ductus vaginalis”. The true vagina is referring to the ones where the vagina is directly connected to the oviduct, while in the “ductus vaginalis” the vagina appears connected to the vitelline ducts (Boeger and Kritsky, 2003). Brooks et al. (1985) considered the presence of bilateral vaginae “ductus vaginalis” as plesiomorphic for monogenea. In contrast, the phylogenetic hypothesis of Boeger and Kritsky (1993) suggests the single ventro-lateral “true” vagina as the plesiomorphic state for the monogenea. Table III4 includes the description of the vagina or vaginal ducts in all the species that comprise the family Macrovalvitrematidae showing the discrepancies among species of the same genus and misinterpretations. Although this has been considered an important taxonomic character, the difficulty in determining the presence or

absence of a vagina and the position and number of vaginal pores makes this as a problematic character that, if any are misinterpreted, could obscure the true relationships among the ingroup. Considering this, it was decided to exclude it from the analysis.

Presence of a fissure in the plate (b) of the ventral valve:

The presence of a plate in the ventral valve of the clamps is a character observed in all the macrovalvitrematids examined. In some, such as the genera *Macrovalvitrematoides*, *Macrovalvitrema* and *Neopteriotrematoides*, a thin fissure is observed in one of the sides of the large clamps, specifically in the inner side (the side facing the other clamp). In the small clamps of *Pseudohargisia* this fissure is also noticeable. This character was removed from the matrix because this character is not distinguishable in the genera of macrovalvitrematids in which the valve width is extremely reduced.

Presence of muscular accessory piece(s) in the border of ventral valve:

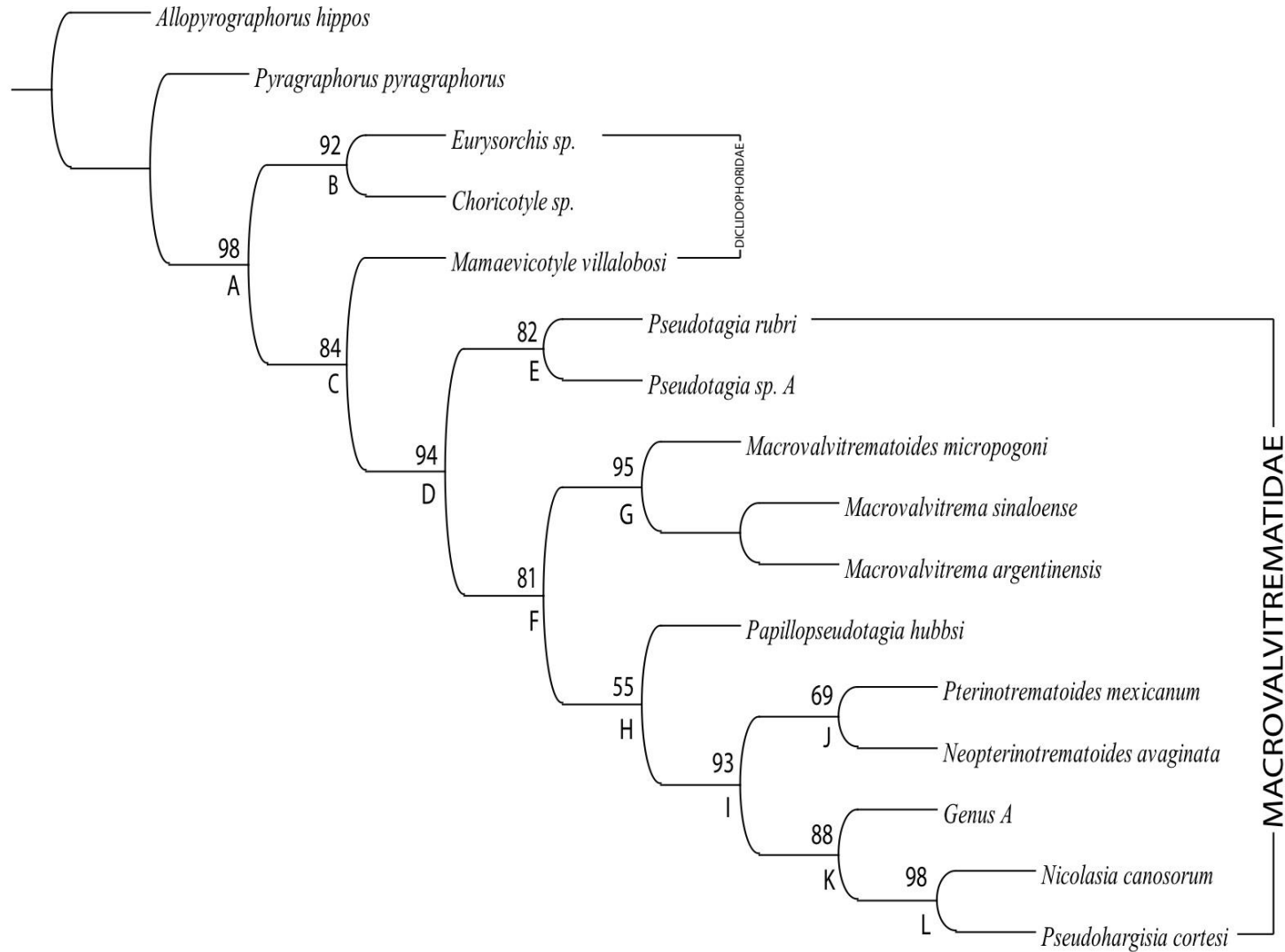
The scalloped muscular pad in the genera *Macrovalvitrematoides*, *Macrovalvitrema*, and the five small papillae along the edge of the ventral valve in the genus *Papillopseudotagia* allow the possibility that these two structures are related by common ancestry. As these structures (scalloped pad, and papillae) are practically an autapomorphy for the genera *Macrovalvitrematoides*, *Macrovalvitrema*, and the genus *Papillopseudotagia* and considering the lack of information about the other species this character was not included.

**Table III-4.** Description of vagina or vaginal ducts in the macrovalvitrematid monogeneans

<b>Macrovalvitrematid species</b>	<b>Reference</b>	<b>Description</b>
<i>Macrovalvitrematoides micropogoni</i>	Payne, 1949 Hargis, 1956	----- Vaginal pores large, on <b>both margins</b> at one fifth level of body, vaginal ducts apparently fusing medially to form common duct which enters vitello-vaginal reservoir anteriorly.
<i>Macrovalvitrema sinaloense</i>	Caballero y C. and Bravo-Hollis, 1955  Bravo-Hollis, 1982 Present Study (Ch. I)	<b>Vagina</b> located at longitudinal median line, between the copulatory organ and the ovary, and opens <b>dorsally</b> , with large vaginal pore.  One <b>vagina</b> opening <b>dorsal</b> with large pore.
<i>Pterinotrematoides mexicanum</i>	Caballero y C. and Bravo-Hollis, 1955  Present study (Ch. I) Bravo-Hollis, 1982	Anterior to the ovary, intercecal and dorsal, opening in a large <b>vaginal pore</b> located at the same level <b>dorsally</b>  Not observed Two laterodorsal vaginal pores
<i>Pseudotagia cupida</i>	Hargis, 1956	<b>Vaginae</b> appearing to open <b>dorsolaterally</b> , often through multiple pores, at one-fourth level of body
<i>Hargisia bairdiella</i>	Hargis, 1956	Pair of antero-lateral plicated placodes interpreted as ornamentation of the region of vaginal openings, because vaginal pores occur in the same position in other species of the genus (referring to the genus <i>Tagia</i> ). <b>Openings and ducts not discernible.</b>
<i>Pseudotagia clathratus</i>	Crane, 1972	<b>Vaginae</b> opening <b>dorso-lateral</b> , a short distance anterior to level of the ovary and join the vitelline ducts.
<i>Macrovalvitrema argentinensis</i> n. comb Syn <i>Neomacrovalvitrema argentinensis</i>	Suriano, 1975  Present study (Ch.I)	Vagina <b>absent</b>  Vagina <b>not observed</b>
<i>Neopteriotrematoides avaginata</i>	Suriano, 1975 Present study (Ch I)	Vagina <b>absent</b> Vagina <b>not observed</b>
<i>Nicolasia canosorum</i>	Suriano, 1975 Present study	Vagina absent Vagina <b>not observed</b>
<i>Pseudohargisia cortesi</i>	Payne, 1987	<b>Vaginal pore dorsomedian</b> , between genital corona and ovary
<i>Papillopseudotagia hubbsi</i>	Payne, 1987	Vagina <b>not observed</b>
<i>Pseudotagia rubri</i>	Luque et al., 1993	<b>Vaginae</b> with <b>laterodorsal</b> pores at genital atrium level
<i>Pseudotagia</i> sp. A	Present study (Ch. I)	Vagina <b>not observed</b>
<b>Genus A</b>	Present study (Ch. I.)	<b>Two vaginae ventrolaterally</b> (one at each side) connected with the vitelline ducts at level of the vittelline reservoir)

**TableIII-5** Matrix of character and character states used for the parsimony and bayesian analysis of the family Macrovalvitrematidae Yamaguti, 1963 (ingroup) and members of Allopyrgraphoridae, Pyrgraphoridae and the family Diclidophoridae (Outgroup) . Unknown characters coded as ? and innapplicables coded as ?.

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	
<i>Allopyrgraphorus hippos</i>	0	0	0	0	2	0	-	1	0	1	1	0	0	1	0	0	-	0	?	0	-	-	?	?	?	?	0	0	-	0	-	0	0	0	0	-	1	-	-	-	-	-	-	-	-	
<i>Pyrgraphorus pyrgraphorus</i>	0	0	0	0	2	?	-	0	0	0	1	0	2	2	1	0	-	0	?	1	-	-	?	?	?	?	0	0	-	0	-	0	0	0	0	-	0	-	-	-	-	-	-	-	-	-
<i>Eurysorchis</i> sp.	0	1	0	0	2	1	1	1	2	0	0	1	0	0	0	0	-	1	1	0	0	0	0	0	0	0	0	?	-	1	-	2	0	0	-	0	-	-	-	-	-	-	-	-	-	-
<i>Choricotyle</i> sp.	0	1	0	1	2	1	1	1	2	1	0	1	1	0	0	0	-	1	1	0	0	0	0	0	0	0	0	1	-	1	-	2	0	0	-	0	-	-	-	-	-	-	-	-	-	-
<i>Mamaevicotyle villalobosi</i>	0	1	1	0	2	1	1	0	1	0	0	1	0	1	0	1	-	1	1	0	0	0	0	0	1	1	0	1	?	0	-	0	0	0	-	0	-	-	-	-	-	-	-	-	-	-
<i>Macrovalvitrematoides micropogoni</i>	1	1	1	1	1	1	1	1	3	1	0	1	1	1	2	1	0	1	?	2	1	0	0	0	?	1	1	2	2	0	0	0	0	0	1	0	1	-	?	?	?	?	?	?	2	
<i>Macrovalvitrematoides sinaloense</i>	1	1	1	1	2	1	1	1	3	1	0	1	1	1	2	1	0	1	1	2	1	0	0	0	2	1	1	2	2	0	0	0	0	0	1	0	1	0	1	1	1	1	1	1	2	
<i>Neomacrovalvitrematoides argentinensis</i>	1	1	1	1	2	1	1	1	3	1	0	1	1	1	2	1	0	1	1	2	1	0	0	0	2	1	1	2	2	0	0	0	0	0	1	0	1	0	1	1	1	1	1	1	2	
<i>Pterinotrematoides mexicanum</i>	1	1	1	1	2	3	0	1	3	1	0	1	1	1	3	1	1	1	1	3	2	0	0	1	?	2	1	2	1	0	1	1	0	0	0	0	0	0	0	1	1	1	1	1	1	1
<i>Neopteriotrematoides avaginata</i>	1	1	1	1	2	3	0	1	3	1	0	1	1	1	3	1	1	1	1	3	2	0	0	1	2	2	1	2	2	0	2	1	0	0	2	0	0	0	1	1	1	1	1	1	1	
<i>Nicolasia hubbsi</i>	1	1	1	0	?	2	1	1	3	1	0	1	1	1	3	1	1	1	?	3	3	2	2	2	1	1	2	2	1	0	3	1	1	0	0	0	0	1	0	0	0	0	0	0	0	
<i>Pseudohargisia cortesi</i>	1	1	1	0	2	1	1	1	3	1	0	1	1	1	3	?	1	1	?	3	3	1	1	2	1	1	1	2	1	0	3	1	1	0	0	0	0	1	0	0	0	0	0	0	0	
<i>Papillopseudotagia hubbsi</i>	1	1	1	1	2	1	1	1	3	1	0	1	1	1	2	1	0	1	1	0	0	0	0	0	2	1	1	1	?	0	-	1	1	0	-	0	0	0	1	1	1	1	0	0	0	
<i>Pseudotagia rubri</i>	1	1	1	1	0	1	1	1	3	0	0	1	0	1	0	1	-	1	1	0	-	-	1	1	1	1	1	2	0	0	-	0	0	0	-	2	-	-	-	-	-	-	-	-	-	
<i>Pseudotagia</i> sp. A.	1	1	1	1	0	1	1	1	3	0	0	1	0	1	0	1	-	1	1	0	-	-	1	1	1	1	1	2	0	0	-	0	0	0	-	0	-	-	-	-	-	-	-	-	-	
Genus A	2	2	2	0	2	3	0	1	3	?	0	1	1	1	3	1	1	1	1	3	2	1	1	2	3	3	1	2	1	0	2	1	0	1	0	0	0	1	0	0	1	0	0	1	0	



**Figure 32.** Phylogenetic hypothesis based on morphological data (43 characters) for the members of the family Macrovalvitrematidae Yamaguti, 1963. Length of tree= 85 steps; Consistency index= 84%; Retention index= 75%. Nodes for each clades indicated by letters in the inferior part of the branch and the bootstrap values (> 50% majority-rule consensus) indicated in the superior part of the branch.

**Table III-6** Synapomorphies and autapomorphies of the hypothesis presented in the Figure?. CI= consistence index of the character, steps= refers to the number of steps corresponding to each character and the transformation = the change of character state.

Synapomorphies / Autapomorphies of:	Character	Steps	IC	Transformation
<b>Clade A</b>	2	2	1.0	0 $\Rightarrow$ 1
	6	4	0.75	0 $\xRightarrow{D}$ 1
	11	1	1.0	1 $\Rightarrow$ 0
	12	1	1.0	0 $\Rightarrow$ 1
	18	1	1.0	0 $\Rightarrow$ 1
<b>Clade B</b>	9	3	1.0	1 $\xRightarrow{A}$ 2
	9	3	1.0	0 $\xRightarrow{D}$ 2
	14	2	1.0	1 $\Rightarrow$ 0
	30	1	1.0	0 $\Rightarrow$ 1
	32	2	1.0	0 $\Rightarrow$ 2
<b>Clade C</b>	3	2	1.0	0 $\Rightarrow$ 1
	16	1	1.0	0 $\Rightarrow$ 1
	24	2	1.0	0 $\Rightarrow$ 2
	26	3	1.0	0 $\xRightarrow{A}$ 1
				0 $\xRightarrow{D}$ 1
<b>Clade D</b>	1	2	1.0	0 $\Rightarrow$ 1
	9	3	1.0	1 $\xRightarrow{A}$ 3
	9	3	1.0	1 $\xRightarrow{D}$ 3
	27	2	1.0	0 $\Rightarrow$ 1
	28	3	1.0	1 $\Rightarrow$ 2
<i>Pseudotagia rubri</i>	36	2	1.0	0 $\Rightarrow$ 2
<b>Clade E</b>	5	2	1.0	1 $\Rightarrow$ 0
<b>Clade F</b>	15	3	1.0	0 $\Rightarrow$ 2
	25	3	0.66	1 $\Rightarrow$ 2
	29	3	0.66	0 $\xRightarrow{A}$ 1
<b>Clade G</b>	20	3	1.0	0 $\Rightarrow$ 2
	21	3	1.0	0 $\Rightarrow$ 1
	35	2	1.0	0 $\Rightarrow$ 1
	37	1	1.0	0 $\xRightarrow{A}$ 1
	42	2	1.0	0 $\xRightarrow{A}$ 1
<i>Macrovalvitrematoides micropogoni</i>	5	2	1.0	2 $\Rightarrow$ 1
<b>Clade H</b>	32	2	1.0	0 $\Rightarrow$ 1
<i>Papillopseudotagia hubbsi</i>	27	2	1.0	1 $\Rightarrow$ 2



**Table II-6. cont.** Synapomorphies and autapomorphies of the hypothesis presented in the Figure?. CI= consistence index of the character, steps= refers to the number of steps corresponding to each character and the transformation = the change of character state.

<b>Clade I</b>	6	4	1.0	1 $\xRightarrow{A}$ 3
	7	2	1.0	1 $\xRightarrow{A}$ 0
	15	3	1.0	2 $\xRightarrow{A}$ 3
	17	1	1.0	0 $\xRightarrow{A}$ 1
	20	3	1.0	0 $\xRightarrow{A}$ 3
	21	3	1.0	0 $\xRightarrow{A}$ 2
	24	2	1.0	2 $\xRightarrow{A}$ 1
	29	3	1.0	0 $\xRightarrow{D}$ 1
	31	3	1.0	0 $\xRightarrow{D}$ 2
<b>Clade J</b>	26	3	1.0	1 $\xRightarrow{A}$ 2
	42	2	1.0	0 $\xRightarrow{A}$ 1
<i>Pterinotrematoides mexicanum</i>	31	3	1.0	2 $\xRightarrow{A}$ 1
<i>Neopterinotrematoides avaginata</i>	35	2	1.0	0 $\xRightarrow{A}$ 2
<b>Clade K</b>	22	2	1.0	0 $\xRightarrow{A}$ 1
	23	3	0.66	1 $\xRightarrow{A}$ 2
	23	3	0.66	0 $\xRightarrow{D}$ 2
	38	1	1.0	0 $\xRightarrow{A}$ 1
	39	1	1.0	1 $\xRightarrow{A}$ 0
	40	1	1.0	1 $\xRightarrow{A}$ 0
<b>Genus A</b>	1	2	1.0	1 $\xRightarrow{A}$ 2
	2	2	1.0	1 $\xRightarrow{A}$ 2
	3	2	1.0	1 $\xRightarrow{A}$ 2
	26	3	1.0	1 $\xRightarrow{A}$ 3
	34	1	uninformative	
<b>Clade L</b>	21	3	1.0	2 $\xRightarrow{A}$ 2
	31	3	1.0	2 $\xRightarrow{A}$ 3
	41	1	1.0	1 $\xRightarrow{A}$ 0
<i>Nicolasia canosorum</i>	6	4	0.75	1 $\xRightarrow{A}$ 2
	22	2	1.0	1 $\xRightarrow{A}$ 2

## Discussion of Characters

The hypothesis (Figure 32) represents the most parsimonious tree (length=85 steps; consistency index= 84%; retention index =88%; homoplasy index=15%) obtained from the parsimony analysis made in PAUP\* PAUP\* and NONA of 42 characters. Clade B is the only that is represented by single diclidophorid genera. All the clades have bootstrap higher than >50%. The strict consensus presents the same topology with the only cladogram obtained by the analysis. Table III-6 lists the synapomorphies and autapomorphies of each clade and taxa of the analysis, and the consistency index obtained by each character.

The analysis that presents a completely resolved tree, suggests the monophyly of the Family Macrovalvitrematidae (Figure 32, clade D) for which include the genera *Pseudotagia*, *Macrovalvitrematoides*, *Macrovalvitrema*, *Papillopseudotagia*, *Pterinotrematoides*, *Neopterinotrematoides*, Genus A, *Nicolasia* and *Pseudohargisia*. The analysis indicates that the goblet-shaped anterior end is wider than the body proper, the entrance of the intestinal cecum into the opisthaptor occurs as one dorsal branch, the lateral sclerites of ventral valve (c and d) fused distally, and the presence of a plate in the dorsal valve extended proximally to both lateral sclerites (c and d) represents synapomorphies for the Macrovalvitrematidae. These synapomorphies correspond respectively to the characters 1, 9, 27 and 28 (Table III6). This is supported by a bootstrap value of 94%, obtained from 1000 replicates in PAUP\*.

The analysis established that *Pseudotagia* is the genus that presents fewer synapomorphies inside the ingroup and situated it as the sistergroup of the common ancestor of the rest of macrovalvitrematid genera. This position in the cladogram is supported by a bootstrap value of

94%. The position of the male copulatory organ anterior to the intestinal bifurcation is the only synapomorphy for the clade composed by the two species of the genus *Pseudotagia* that were examined.

The rest of macrovalvitrematid genera including *Macrovalvitrematoides*, *Macrovalvitrema*, *Papillopseudotagia*, *Pterinotrematoides*, *Neopterinotrematoides*, *Nicolasia*, *Pseudohargisia* and Genus A are suggested to be evolved from a more recent common ancestor than *Pseudotagia*. This clade (Figure 32 Clade F) is supported by a bootstrap value of 81% and by two additional synapomorphies, the presence of two kinds of clamps (three pair of large clamps and one pair of small clamps) and a sclerite f (median sclerite) with a curved basal end (Characters 15, 25 respectively).

The clade G or the clade that refers to the most recent common ancestor for the macrovalvitrematids of the genera *Macrovalvitrematoides*, *Macrovalvitrema* and *Neomacrovalvitrema* is supported by a bootstrap value of 95% and five synapomorphies. These correspond to a slight strangulation of the lateral sclerites (Character 20, Figure 13B), the lateral sclerites of the dorsal valve ( $g_1$  and  $g_2$ ) with semicircle-shape (Character 21, Figure 15B), the presence of asymmetric accessory sclerites at strangulation level (Character 35, Figure 27B), the presence of strangulation in the pair of small clamps (Character 37, Figure 28B) and the extend of the plate along the complete ventral valve (Character 42, Figure 31C).

The morphology of the clamps of these three genera maintains an identical arrangement. None of the other macrovalvitrematid maintains identical clamp morphology with their sistergroups. Also

the only character of this analysis that separate the genus *Macrovalvitrematoides* from the other two genera is the position of the male copulatory which appears to be at level of the intestinal bifurcation in *Macrovalvitrematoides micropogoni*, character state suggested in the hypothesis as an autapomorphy for this species.

Other difference between *Macrovalvitrematoides* and the genera *Macrovalvitrema* and *Neomacrovalvitrema* is the position of the vagina, which according to the description of Hargis, 1956 has two large vaginal pores, one in each margin of the body. In the microphotographs provided by the USNPC the position of these are not clearly visible. Also the most significant difference that Suriano, 1975 established for *Neomacrovalvitrema argentinensis* with respect to *Macrovalvitrema sinaloense* was the absence of vagina. See Chapter I Taxonomic Note and Remarks in the description of *Macrovalvitrema argentinensis* for a discussion of the vagina).

The position of *Papillopseudotagia hubbsi* as sister group of the macrovalvitrematids that compose the clade I ((*Pterinotrematoides*, *Neopterinotrematoides*, (Genus A, (*Nicolasia*, *Pseudohargisia*))) of the cladogram (Figure 32) resulted in the lower bootstrap value (55%) of the hypothesis obtained from this parsimony analysis. This is supported by one synapomorphy obtained with both optimization (ACCTRAN and DELTRAN), the presence of bars in the dorsal valve (Character 32). In addition to this synapomorphy the cladogram suggests that the absence of strangulation in the small pair of clamps and the extend of the plate beside the sclerite c of the ventral valve of the small pair of clamps (Characters 37 and 42 and Figures 28A and 31A respectively) related *Papillopseudotagia hubbsi* with their sister group (Clade I) that includes the genera *Pterinotrematoides*, *Neopterinotrematoides*, Genus A, *Nicolasia* and *Pseudohargisia*.

These two character states correspond or is suggested in the cladogram as the plesiomorphic condition which are found derived and forming a synapomorphy respectively in the clades (*Macrovalvitrematoides*, (*Macrovalvitrema sinaloese*, *Neomacrovalvitrema argentinensis*)) and (*Pterinotrematoides*, *Neopterinotrematoides*). The condition of having the plate beside the sclerite c is also observed and maintained as plesimorphic state in the pairs of large clamps (see character 28, Figure 21B).

The clade I (Figure 32) corresponding to ((*Pterinotrematoides*, *Neopterinotrematoides*, (Genus A, (*Nicolasia*, *Pseudohargisia*)) represents the clade best supported of the analysis with a bootstrap value of 93% and seven synapomorphies. This includes:

- ⊃ The derived condition of two kinds of clamps in which the pair of small clamps has different frame and arrangement without the strangulation that characterize the large clamps.
- ⊃ The position of small pair of clamps posterior to the three large pairs of clamps.
- ⊃ The conspicuous strangulation at midline level of the clamp (Character 20, Figure 13C).
- ⊃ The butterfly shape of the sclerites  $g_1$  and  $g_2$  of the dorsal valve (Character 21, Figure 15).
- ⊃ The slightly curved distal end of the sclerite f (median sclerite).
- ⊃ The development of cardioid shape in the sclerites c and d of the ventral valve (Character 31, Figure 23C).
- ⊃ In addition to these, the slow optimization (DELTRAN) suggest that extend of the plate of the ventral valve to the strangulation level is another synapomorphy for this clade, but the fast optimization (DELTRAN) suggests the establishment of this synapomorphy in the clade F.

The clade composed of the genera *Pterinotrematoides* and *Neopterinotrematoides* (Figure 32 Clade J) is supported by a bootstrap value of 69% and two synapomorphies that includes the U-shaped basal sclerite (Character 26, Figure 19C) and the plate of small clamps medially positioned and extended to median level of the clamp (Character 42, Figure 31B). Apart to these synapomorphies, the large clamps of *Neopterinotrematoides* presents some characteristics similar to those found in the clade composed by the genera *Macrovalvitrematoides*, *Macrovalvitrema* and *Neomacrovalvitrema* as the plate extended to distal segment of the ventral valve and the presence of accessory sclerites at strangulation level. But the accessory sclerites of this are symmetric while the ones found in *Macrovalvitrematoides*, *Macrovalvitrema* and *Neomacrovalvitrema* are asymmetric. *Neopterinotrematoides* also has the fissure of the plate observed in the clamps of *Macrovalvitrematoides*, *Macrovalvitrema* and *Neomacrovalvitrema*. However, this character was not included in the analysis because the reduction in the width of proximal segment of the clamps, the fissure was not distinguishable in all macrovalvitrematids.

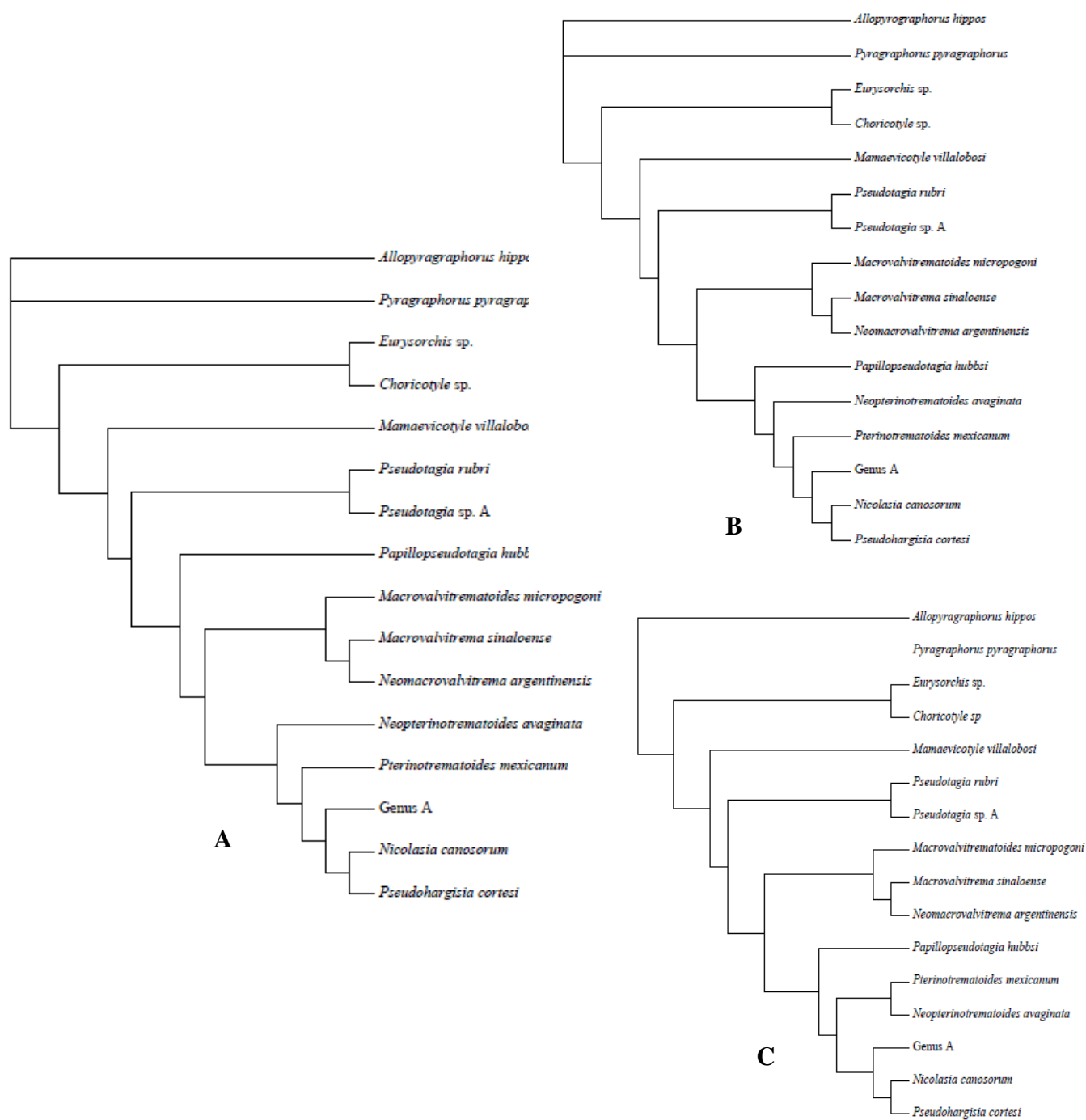
The position of the Genus A as the sister group of (*Nicolasia*, *Pseudohargisia*) (Figure 32, Clade K) is another well supported clade with a bootstrap of 88% and five synapomorphies corresponding to the presence of an appendix of the sclerite  $g_1$  and  $g_2$  of dorsal valve (Character 22, Figure 16), the distally fused condition of the sclerites  $g_1$  and  $g_2$  of the small pair of clamps (Character 38, Figure 29B), the distal end of the sclerite f (median sclerite) with the condition of being distant to the edge of the sclerites  $g_1$  and  $g_2$ , in both the large and

small pair of clamps (Characters 23 and 39, Figure 17B and 29A), the slightly curved distal end of the sclerite f (median sclerite) in the small pair of clamps (Character 40, Figure 30B).

The clade (*Nicolasia*, *Pseudohargisia*) (Figure 32 Clade L) occupy the most inclusive position of the cladogram. This is well supported by a bootstrap value of 98% and three synapomorphies including the derived condition of laterally extended butterfly shape in the sclerite c and d of both valves (Characters 21 and 31, Figures 15D and 23D) respectively for the dorsal and ventral valves, and the basal end of median sclerite curved toward down or posteriorly (Character 41).

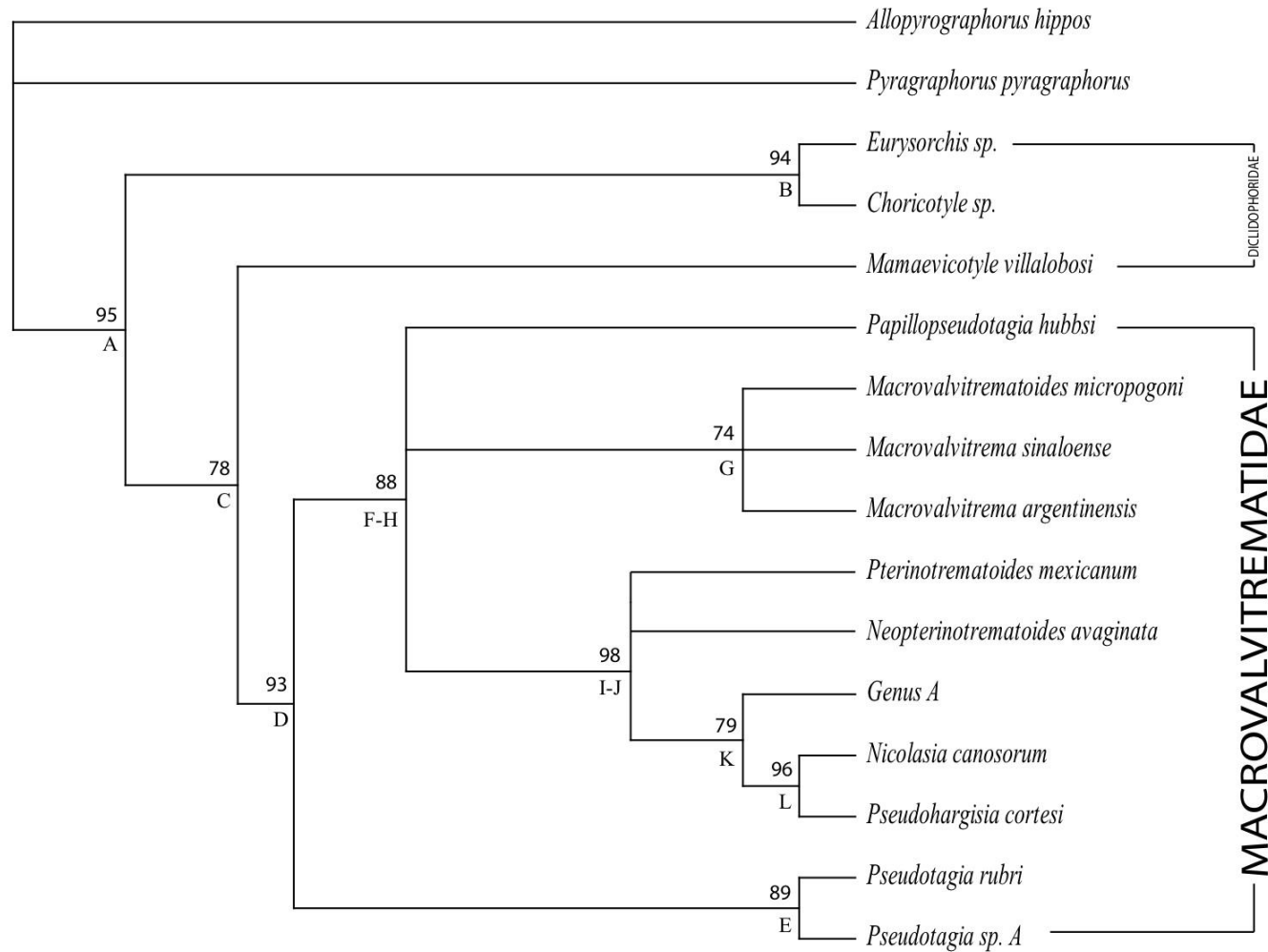
### **Status of Diclidophoridae**

The analysis also propose the paraphyly of Diclidophoridae (Figure 32). This is shown by the clade C which established the diclidophorid *Mamaevicotyle villalobosi* as sister group of the macrovalvitrematids and this is supported by the following synapomorphies: 1. Oral suckers larger than the pharynx, 2. presence of a lappet, 3. curved basal end of the sclerite f (median sclerite) and 4. “pelvic ring-shaped” basal sclerite without median sclerite ( $a_1$ ). The paraphyletic status of Diclidophoridae conferred by Macrovalvitrematidae was suggested by Mamaev (1976), argument used by Boeger and Kritsky (1993) to situate the Macrovalvitrematidae in the Family Diclidophoridae in the revised classification of the Class Monogenea. The clade (*Eurysorchis* and *Choricotyle*) (Figure 32, clade B) is supported by a bootstrap value of 92% and five synapomorphies (Table III-5, clade B) that includes one intestinal branch extended to each peduncle of the opisthaptor, a round diclidophorid kind of clamp, the presence of a diaphragm in the ventral loop (valve) of the clamps and the rib-like ornamentation of the dorsal loop (valve).



**Figure III-33.** Three cladograms with the highest posterior probability of 127 cladograms constructed using Bayesian analysis of Family Macrovalvitrematidae. The posterior probability of hypothesis **A** was of 0.045 and **B** and **C** had a posterior probability of 0.035.





**Figure III-34.** Consensus tree (50% majority-rule) constructed from the 127 trees resulting from Bayesian analysis based on morphological data (43 characters) for the members of the family Macrovalvitrematidae Yamaguti, 1963. Nodes for each clade are indicated by letters on the lower side of the branches and the percentage of the posterior probabilities are indicated on the upper side of the branches.

## Discussion of Bayesian analysis

The topology of the three cladograms with the highest posterior probabilities resulting from the Bayesian analysis (Figure 33) is similar to the hypothesis resulting from the parsimony analysis, suggesting the monophyly of the Macrovalvitrematidae, the basal position for the genus *Pseudotagia* and the paraphyletic status of the Diclidophoridae are preserved. Also preserved is the exact topology of the cladogram resulting from parsimony for the clades K (Genus A, (*Nicolasia*, *Pseudohargisia*)) and L (*Nicolasia*, *Pseudohargisia*).

The cladogram with the highest posterior probability (0.045) found during the MCMC search (Figure 33A) differed from the cladogram resulting from parsimony by the switching of positions of *Papillopseudotagia hubbsi* with the clade (*Macrovalvitrematoides*, (*Macrovalvitrema*, *Neomacrovalvitrema*)). This hypothesis (Figure 33A) related all the macrovalvitrematids that have some level of strangulation in the large clamps with the proposal of the clade ((*Macrovalvitrematoides*, (*Macrovalvitrema*, *Neomacrovalvitrema*))), (*Neopterinotrematoides*, (*Pterinotrematoides*, (Genus A, (*Nicolasia*, *Pseudohargisia*)))) and placed *Papillopseudotagia* in a basal position with respect to these.

Another difference between this hypothesis (Figure 33A) and the one resulting from the parsimony analysis is the relationship between the genera *Neopterinotrematoides* and *Pterinotrematoides*. Unlike the parsimony analysis, the Bayesian analysis suggests *Neopterinotrematoides* has a more basal position than *Pterinotrematoides*, placing the genus *Pterinotrematoides* in a clade with (Genus A, (*Nicolasia*, *Pseudohargisia*)).

Figures 33 B and C present two other trees with the next highest posterior probabilities among the 127 (posterior probability of 0.035 for each) that were found during the search of the mcmc. These two cladograms suggest the same topology for the clade (*Macrovalvitrematoides*, (*Macrovalvitrema*, *Neomacrovalvitrema*)) and for *Papillopseudotagia* as did the parsimony hypothesis. These two differ between each other in the relationship between *Neopterintrematoides* and *Pterintrematoides*. The hypothesis of the Figure 33B has an identical topology of the tree resulting from parsimony suggesting that these two genera share a clade (Figure 32, Clade I). The Figure 33 C suggests the same arrangement for these two genera as did the tree in Figure 33A (with highest posterior probability of the bayesian analysis).

Figure 34 presents a strict consensus in which the credibility values assigned to each clade are included. In this figure the monophyly of the Macrovalvitrematidae (Clade D) and the basal position of the genus *Pseudotagia* among the macrovalvitrematids are shown and have a credibility value (posterior probability) of 98%. This is similar to the support found in the parsimony analysis (bootstrap value 94%). The consensus tree resulted in a few collapsed clades producing a polytomy of the clade (*Macrovalvitrematoides*, (*Macrovalvitrema*, *Neomacrovalvitrema*)); the clade (*Neopterintrematoides*, *Pterintrematoides*, (Genus A, (*Nicolasia*, *Pseudohargisia*))); and *Papillopseudotagia*.

Other polytomies included the branches leading to the genera *Pterintrematoides* and *Neopterintrematoides*, and the clade (Genus A, (*Nicolasia*, *Pseudohargisia*)). The parsimony analysis suggests that the genera *Pterintrematoides* and *Neopterintrematoides* are related in a clade supported with a 69% bootstrap value and two synapomorphies that corresponds to a U-

shaped basal sclerite and the plate of the pair of small clamps medially positioned reaching the median level of the valve.

## Phylogeny and Revised classification of Macroalvitrematidae Yamaguti, 1963

A revised classification for the Family Macroalvitrematidae is proposed based on the results of the phylogenetic analysis of 42 characters using parsimony and Bayesian methods; the analysis of the character transformation along the cladogram; and other morphological characters not analyzed. The diagnoses include cladistic and traditional information.

This classification proposed for the family Macroalvitrematidae Yamaguti, 1963 includes:

- ⊃ Nine genera and 14 species recognizing the genera *Macroalvitrema*, *Pterinotrematoides*, *Neopterinotrematoides*, *Nicolasia*, *Pseudotagia* and *Papillospseudotagia*
  - ⊃ Recognition of the genera *Macroalvitrematoides* and *Hargisia* but with the recommendation of further evaluation, considering that only several photomicrophotographs of the holotype of *Macroalvitrematoides* were examined provided by the USNPC and that it was not possible to examine the material of *Hargisia* because it is no longer available in the USNPC.
  - ⊃ Recognition of the material collected from Puerto Rico (Caribbean, western Atlantic) from the seabream *Archosargus rhomboidalis* as a new genus and species presenting several autapomorphies and distinctive character states.
- ⊃ Reassign of the genera *Neomacroalvitrema* and *Pseudohargisia* to the genera *Macroalvitrema* and *Nicolasia* respectively with the creation of new combinations:
  - ⊃ *Macroalvitrema argentinensis* (Suriano, 1975) synonym: *Neomacroalvitrema argentinensis* Suriano, 1975



evolution of the Macrovalvitrematidae from the parsimony and bayesian analyses. Figure 35 presents the relationships among the four subfamilies proposed in this classification.

The subfamily new A comprises distinct basal lineages formed by the genus *Pseudotagia*, this is suggested by both competing hypotheses. The composition and the position of the subfamily Pterinotrematoidinae as a terminal clade is equally suggested by both competing hypotheses. The position of the taxa or taxon that represents the subfamilies Macrovalvitrematinae (*Macrovalvitrematoides*, *Macrovalvitrema* and *Neomacrovalvitrema*) and the subfamily new B (*Papillopseudotagia*) respectively is the main controversial issue of the phylogenetic hypotheses presented by the two analyses (See Figures, 32 and 33A-C), but both hypotheses are consistent in supporting them as separate branches.

Payne (1987) includes *Papillopseudotagia* in the subfamily Macrovalvitrematinae Bravo-Hollis (1982) because it has all the clamps similar suggesting similarities of the clamps with *Pseudotagia*. The only similarity observed between the clamps of *Papillopseudotagia* and *Pseudotagia* is the absence of strangulation which is suggested by the analysis as ancestral condition. *Pseudotagia* were included in the subfamily Macrovalvitrematinae by Bravo-Hollis (1982) because all four pairs of clamps are similar in structure and the copulatory organ (referred as genital atrium) has spines arranged radially. These two genera were removed from the subfamily Macrovalvitrematoidinae based on the amended diagnosis (below).

The character analysis, does not suggest differences that distinguishes *Neomacrovalvitrema argentinensis* Suriano, 1975 from *Macrovalvitrema sinaloense*. The examination of the holotype

material and new material collected from Puerto Rico of *N. argentinensis* present the number of testes and the number of copulatory organ spines as different between these two. These differences are not enough to separate them into two genera. Suriano (1975) used the “absence of vagina” as the only distinctive character to establish the genus *Neomacrovalvitrema*. Although the vagina was not observed in any of the specimens examined in this study and, considering that this is a very controversial character that is difficult to see in many monogeneans, in this study it is proposed that the “apparent absent of the vagina” is not sufficient to place this species in a separate genus. This study proposes the new combination *Macrovalvitrema argentinensis* comb. nov. (Suriano, 1975).

Payne (1987) emended the subfamily Pterinotrematoidinae to include the male copulatory organ armed with a radially arranged corona of spines (as observed in *Pseudohargisia*) along with the male copulatory organ with two lateral groups of dissimilar spines (as observed in *Pterinotrematoides* and *Neopterinotrematoides*). This is accepted because the hypotheses support the clade referring to this subfamily that includes these two kinds of copulatory organs. Payne (1987) did not compare their material (referring to *Pseudohargisia cortesi*) with *Nicolasia canosorum*. According to the character analysis and the hypotheses these two species are closely related with few differences between them and many synapomorphies in common, thus it is proposed that both belong to the same genus, suggesting the new combination: *Nicolasia cortesi* comb. nov. (Payne, 1987).

*Hargisia bairdiella* is tentatively maintained in the subfamily Pterinotrematoidinae Bravo-Hollis, 1982 based only in the drawings and descriptions made by Hargis (1956) and Yamaguti (1963)



and the position assigned by Bravo-Hollis (1982). The same occurs with *Pseudotagia cupida* which is tentatively located in the Subfamily New A based in the previous publications (mentioned for *H. bairdiella*), but these two species needs to be newly collected and further examined to determine their position among the other macrovalvitrematids and in the Family Macrovalvitrematidae. The USNPC have lost in another loan the type material of these two species together with the paratype material of *Macrovalvitrematoides micropogoni* collected all by Hargis (1956).

*Macrovalvitrematoides micropogoni*, anterior to Hargis (1956) have been already collected and reported by Pearse (1949) but their description was incomplete, which is the reason why Hargis (1956) redescribed the species using the holotype material (collected by Pearse, 1949) and new material. Although the slides of the material collected by Hargis (1956) were lost, and the holotype material deposited by Pearse (1949) was not studied in the present work, the museum provided several photographs instead of loaning the holotype. These pictures of the opisthaptor , clamps, anterior end, genitalia and complete body were included in the analysis. Some parts or structures were not clearly observed in the pictures. Considering this, this species also needs to be newly collected and reexamined.

## Summary of the Revised Taxonomic Classification proposed for Macrovalvitrematidae

Family Macrovalvitrematidae Yamaguti, 1963

Subfamily new A

Genus *Pseudotagia* Yamaguti, 1963

*Pseudotagia cupida* (Hargis, 1956) Yamaguti, 1963

Synonym: *Tagia cupida* Hargis, 1956

*Pseudotagia clathratus* Crane, 1972

*Pseudotagia rubri* Luque et al., 1993

*Pseudotagia* new species A

Subfamily Macrovalvitrematinae Bravo-Hollis, 1982

Genus *Macrovalvitrematoides* Yamaguti, 1963

*Macrovalvitrematoides micropogoni* (Hargis, 1956) Yamaguti, 1963

Synonym: *Tagia micropogoni* Hargis, 1956

Genus *Macrovalvitrema* Caballero y C. and Bravo-Hollis, 1955

*Macrovalvitrema sinaloense* Caballero y C. and Bravo-Hollis, 1955

*Macrovalvitrema argentinensis* new combination (Suriano, 1975)

Synonym: *Neomacrovalvitrema argentinensis* Suriano, 1975

Subfamily new B

Genus *Papillopseudotagia* Payne, 1987

*Papillopseudotagia hubbsi* Payne, 1987

Subfamily Pterinotrematoidinae Bravo-Hollis, 1982

Genus *Pterinotrematoides* Caballero y C. and Bravo-Hollis, 1955

*Pterinotrematoides mexicanum* Caballero y C. and Bravo-Hollis, 1955

Genus *Neopteriotrematoides* Suriano, 1975

*Neopteriotrematoides avaginata* Suriano, 1975

Genus new A

Genus *Nicolasia* Suriano, 1975

*Nicolasia canosorum* Suriano, 1975

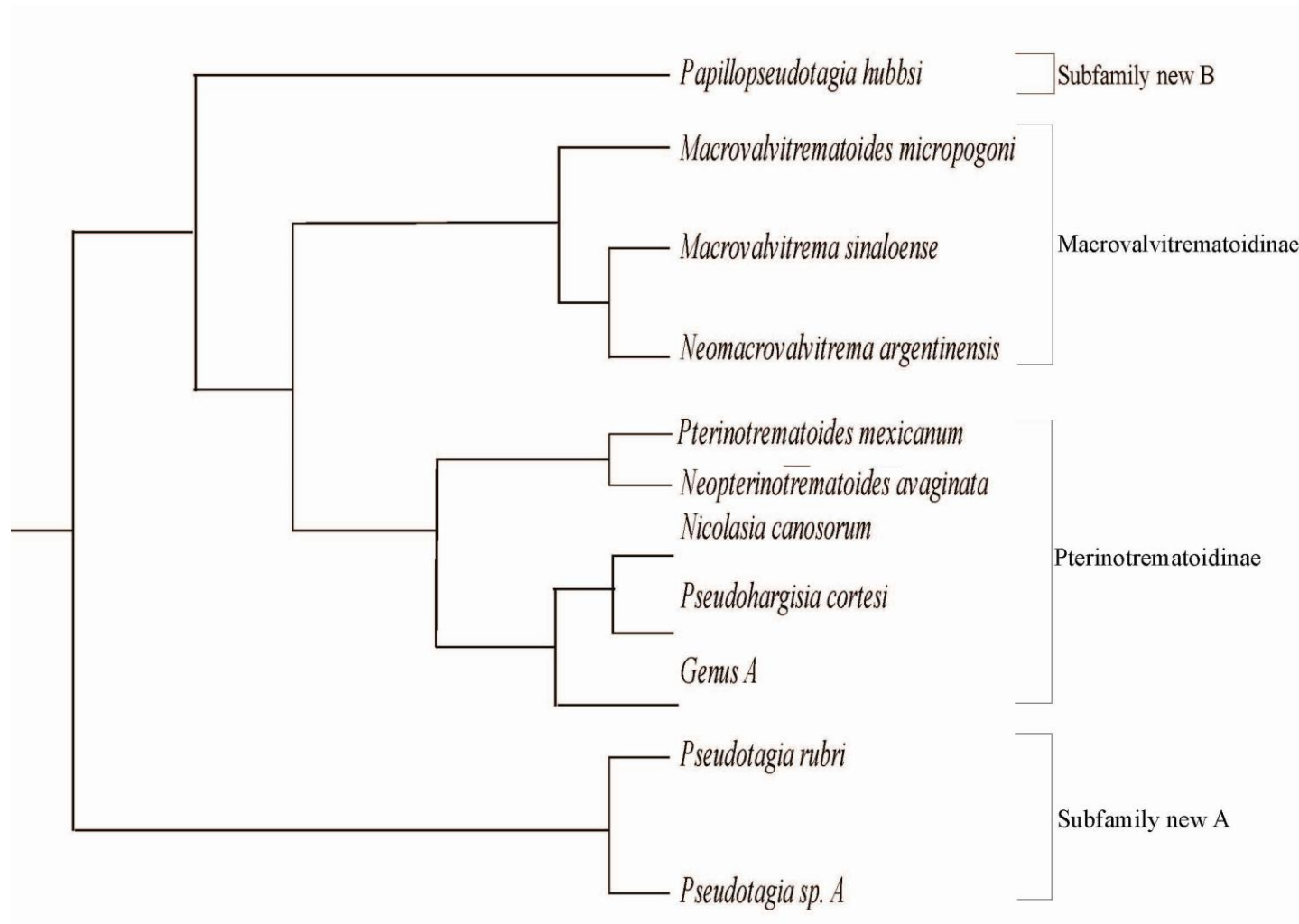
*Nicolasia cortesi* new combination (Payne, 1987)

Synonym: *Pseudohargisia cortesi* Payne, 1987

Genus *Hargisia* Yamaguti, 1963

*Hargisia bairdiella* (Hargis, 1956) Yamaguti, 1963

Synonym: *Tagia bairdiella* Hargis, 1956



**Figure III-35.** Cladogram representing the hypothesis resulting from the parsimony analysis of 11 taxa of the family Macrovalvitrematidae based in 42 morphological characters. Length of tree= 85 steps; Consistency index= 84%; Retention index= 75%. The brackets are indicating the monophyletic groups considered as subfamilies.

### **Diagnoses and Amendments to previous diagnoses**

The complete diagnoses was only included for the family and for the new two subfamilies proposed (Subfamily new A and Subfamily new B) and for the Genus A. For the other divisions inside the subfamilies, only the amendments were included. The plesiomorphic characters of the Macrovalvitrematidae will be presented in bold in the diagnosis of the family. The autapomorphies for the subfamilies and genera will also be presented in bold in their respective diagnoses.

### **Family Macrovalvitrematidae Yamaguti, 1963**

**Diagnosis:** Body cylindrical with **goblet-shaped anterior end** of the same size or wider than body proper. Having a terminal or subterminal mouth with one pair of muscular oral suckers with or without slightly sclerotized edges. **Presence** or absence of a pharynx, when **present** size smaller than oral suckers located between and posterior to them. Oral disc with or without glands. Intestinal caecae with ramifications. extending along body proper and **rejoining posterior** to the testes then entering opisthaptor in a **single dorsal branch**. Male copulatory organ armed with a **corona of spines arranged radially** or bilaterally symmetrical located at level of or **posterior to** intestinal bifurcation . Posterior part of body proper constricted or **not constricted**, distinguishing the opisthaptor. Opisthaptor symmetrical, armed with four pairs of asymmetrical clamps on a **short** or long peduncles, possessing a lappet located between posteriormost pair of clamps, armed with two pairs of hooks. Clamps are longer than wide with or **without strangulation** at median level, shaped like a miter, fire tongs or guitar. Having **four pairs of similar clamps** or two types of clamps, each pair a mirror image of the other. Clamps with 8 sclerites, five in dorsal valve, two in ventral valve and one basal sclerite. Ventral valve

characterize by presence of a plate and presence or absence of accessory sclerites at strangulation and proximal level. Testes postovarian variable in numbers, ovary curved anterior to testes, vagina present or not visible. Vitellaria co-extensive with intestine along body.

Type species: *Macrovalvitrema sinaloense* Yamaguti, 1963

### **Subfamily new A**

**Type and only Genus:** *Pseudotagia* Yamaguti, 1963

**Diagnosis:** Body cylindrical and wide, with opisthaptor wide, armed with four pairs of similar clamps. All clamps have uniform structure with size only slightly variable. Clamps without strangulation, with two **valves covered with slight lamina**. Lateral sclerites of clamps with or without **serration**. Lateral sclerites of both clamp valves fused distally. Both dorsal and ventral valves have thin lateral sclerites. **Median sclerite of dorsal valve reforced laterally** and plate of the ventral valve small located in proximal region. **Dorsal valve with pair of accessory sclerites oriented toward ventral valve**. Basal sclerite large (pelvic-ring-shaped). Anterior end of body goblet shaped with two muscular oral suckers. Small pharynx present with short esophagus. Intestinal bifurcation posterior of male copulatory organ, with cecae rejoining posteriorly or not. Numerous testes posterior to ovary. Male copulatory organ armed with six to eight radially arranged spines. Without constriction separating opisthaptor from body proper. Vaginae present opening dorsolaterally with ovary curved anterior to testes. Eggs with one round pole and one fusiform pole with long filament.

*Pseudotagia rubri* Luque et al., 1993

**Amendments to diagnosis:** One median sclerite located in dorsal valve instead of two median sclerites, one on each side. Lateral sclerites of dorsal valve thin bordered by a thin lamina giving the false appearance of being thick.

*Pseudotagia* sp. A

\*Their description is in Chapter I.

**Subfamily Macrovalvitrematinae** Bravo-Hollis, 1982

**Type genus** *Macrovalvitrema* Caballero y C. and Bravo-Hollis, 1955

**Amendments to diagnosis:** Four pairs of clamps with similar frame, and **slight strangulation** at median level. Possessing **two types of clamps: three larger pairs with scalloped pad delimited by three sclerotized pieces at edge of ventral valve;** and one small pair positioned anteriorly with similar sclerite arrangement as large pairs but lacking scalloped pad at the edge of ventral valve. Clamps with evident asymmetry, composed of eight sclerites, five in the dorsal valve, two in the ventral valve and one basal sclerite (pelvic ring-shaped). Dorsal valve with anterior segment of **lateral sclerites semicircular shaped**, median sclerite well developed reaching or exceeding distal edge of lateral sclerites. Ventral valve with large plate extending almost the complete length of valve, surrounded by muscular wall, possessing **a pronounced suture at one side of plate**. Opisthaptor armed with larval lappet located between posteriormost pair of clamps with two pair of hooks.

**Subfamily Pterinotrematoidinae** Yamaguti, 1963

**Type genus:** *Pterinotrematoides* Caballero y C. and Bravo-Hollis, 1955

**Amendments to diagnosis:** Opisthaptor with four pairs of clamps of two types: **three pairs of large raquet-shaped clamps**, with **prominent strangulation at longitudinal midline**; and **one pair of small clamps without strangulation positioned posterior to larger pair of clamps**.

Clamps asymmetrical with each pair positioned as mirrow images of the other. Clamps with eight sclerites, five in the dorsal valve, two in the ventral valve and one dorsal valve pelvic ring-shaped, U-shaped or U-shaped fused to the lateral sclerite of ventral valve. The dorsal valve of large clamps with distal section of lateral sclerites **butterfly-shaped or laterally butterfly-shaped** with or without **appendix** and median sclerite with slightly curved distal ends, distant from distal edge of lateral sclerites. Lateral sclerite of ventral valve with an oval, cardioid or butterfly shape. Ventral valve with a plate extending toward distal part of valve or toward the strangulation level in large clamps. Accessory sclerites present or absent at strangulation level of ventral valve . Pair of small clamps with plate of ventral valve alongside one of the lateral sclerites.

### **Genus new A**

**Generic diagnosis:** Pterinotrematoinae. Body elongate, narrow with **wide goblet-shaped anterior end, twice the width of body proper**. Anterior end with a large oral disc and large, **rectangular oral suckers with slightly sclerotized borders, pharynx absent**. Copulatory organ armed with five spines, one large medially located and two smaller pairs arranged bilaterally symmetrical. Ovary curved, two vaginal pores opening ventrolaterally at vitelline reservoir. Opisthaptor armed with three pairs of large racket-shaped clamps, one pair of small clamps with lappet located between and posterior. Large pair of clamps with **lateral short spines in both**



**valves**, appendix extended from the lateral sclerites of dorsal valve, **basal sclerite U-shaped fused ventrally with lateral sclerite**, plate of dorsal valve extended to strangulation level. Small pair of clamps without strangulation, with lateral sclerites of dorsal valve fused distally and plate of ventral valve limited to side of one lateral sclerite.

*Nicolasia* Suriano, 1975

**Type species:** *Nicolasia canosorum* Suriano, 1975

**Amendments to diagnosis:** Lateral sclerites of dorsal and ventral valves laterally extended butterfly-shaped and with appendix of distal segment of lateral sclerites of dorsal valve present.

### **Subfamily new B**

**Type Genus:** *Papillopseudotagia* Payne, 1987

**Diagnosis:** Macrovalvitrematid with wide goblet-shaped anterior bearing large oral suckers and very small pharynx. Male copulatory organ located posterior to intestinal bifurcation, armed with a corona of radially arranged spines. Opisthaptor with four pairs of clamps of similar structure but differing in size. Three pairs of larger clamps with five **papillae** located at distal edge of ventral valve. One pair of smaller clamps positioned anteriorly similar to larger pairs but lack characteristic papillae on ventral valve. Clamps without strangulation possessing eight sclerites; five in dorsal valve, two in ventral valve and one **modified pelvic ring shaped basal sclerite inclined more toward one side than the other**. Dorsal valve with very short lateral sclerites and thin median sclerite extending to distal edge of lateral sclerite. **Ventral valve with plate oriented along one lateral side. Both valves with several rows of bars**. Opisthaptor also with larval lappet between posteriormost pair of clamps.

### Literature Cited

- Boeger, W. A. and D. C. Kritsky. 1993. Phylogeny and a revised classification of the Monogenoidea Bychowsky, 1937 (Platyhelminthes). *Systematic Parasitology* 26: 1-32.
- Boeger, W. A. and D. C. Kritsky. 1997. Coevolution of the Monogenoidea (Platyhelminthes) Based on a Revised Hypothesis of Parasite Phylogeny. *International Journal for Parasitology* 27: 1495—1511.
- Boeger, W. A. and d. C. Kritsky. 2001. Phylogenetic Relationships of the Monogenoidea. *In* Interrelationships of the Platyhelminthes, D. T. J. Littlewood and R. A. Bray (eds.). Taylor and Francis, London, U. K., p. 92-102.
- Bravo-Hollis, M. 1982. Helmintos de peces del pacific mexicano XXXIX. Dos subfamilias nuevas de Monogéneos de la familia Macrovalvitrematidae Yamaguti, 1963. *Anales del Instituto de Biología Universidad Nacional Autónoma de México* 52: 27-38.
- Brooks, D. R. 1989. The phylogeny of the cercomeria (Platyhelminthes: Rhabdocoela) and general evolutionary principles. *Journal of Parasitology* 75: 606-616.
- Brooks, D. R., R. T. O'Grady & D. r. Glenn. 1985. The phylogeny of the cercomeria Brooks, 1982 (Platyhelminthes). *Proceedings of the Helminthological Society of Washington*, 52: 1-20.
- Buchman, K. and T. Lindstrom. 2002. Interactions between monogenean parasites and their fish hosts. *International Journal for Parasitology* 32: 309-319.
- Bychowsky, B. E. 1957. Monogenetic trematodes, their systematics and phylogeny. Engl. Transl.: American Institute of Biological Sciences of Washington.
- Caballero y C. y Bravo-Hollis, 1955. Trematodos de peces marinos de aguas mexicanas del Océano Pácifico. VIII. Descripción de tres nuevos generous de trematodos monogéneos. *Anales del Instituto de Biología* 16: 89-115.
- Crane, J. W. 1972. Systematics and New Species of Marine Monogenea from California. *The Wasmann Journal of Biology* 30: 109-166.
- Ehlers, U. 1985. *Das phylogenetische System der Platyhelminthes*. Stuttgart: Gustav Fisher Verlag, 317 pp.
- Euzet, L. And M. Prost. 1981. Report of the meeting on the “Monogenea” problems of systematic, biology and ecology”. Pages 1003-1004. *In*: W. Slusarki (Ed. ) Review of \ advances in Parasitology. PWN-Polish Scientific Publishers, Warsaw, Poland.

- Glennner H., A. J. Hansen, m. V. Sorensen, F. Ronquist, J. Huelsenbeck and E. Willerslev. 2004. Bayesian inference of the metazoan phylogeny: a combined molecular and morphological approach. *Current Biology* 14: 1644-1649.
- Hargis, W. J. Jr. 1956. Monogenetic Trematodes of Gulf of Mexico Fishes. Part VIII. The Superfamily Diclidophoroidea Price, 1936. (Continued). *Helminthological Society* 23: 5-13.
- Hernández-Vale, O. 2003. Heteronchoinean monogenea from the gills of some fishes from Puerto Rico. University of Puerto Rico, Mayagüez Campus Master Thesis, 193 pp.
- Justine, J. L. Cladistic study in the Monogenea (Platyhelminthes) based upon a parsimony analysis of spermiogenetic and spermatozoa ultrastructural characters. *International Journal for Parasitology* 21: 821-838.
- Justine, J. L. Phylogenie des Monogenes basee sur une analyse de parcimonie des caracteres d l'ultrastructure de la spermigenenese et des spermatozoids incluant les resultants recents. *Bull. Fr Peche Pisc* 328: 137-155.
- Justine J. L. 2001. Spermatozoa as phylogenetic characters for the Platyhelminthes In: Littlewood D. T. J., Bray R. A. *Interrelationships of the Platyhelminthes*, London: Taylon and Francis.
- Lewis, P. O. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology* 50: 913-925.
- Littlewood, D. t. J. and P. D. Olson. 2001. Small subunit rDNA and the Platyhelminthes: signal, noise, conflict and compromise. In: Littlewood, D. T. J. Bray, R. A (Eds.) *Interrelationships of the Platyhelminthes*, Taylor & Francis, London, pp. 267-278.
- Llewelyn J. 1958. The adhesive mechanisms of monogenetic trematodes: the attachment of species of the Diclidophoridae to the gills of gadoid fishes. *Journal of the Marine Biological Association of the United Kingdom* 37: 67-79.
- Luque, J. L., J. F. R. Amato and R. M. Takemoto. 1993. A New Species of *Pseudotagia* Yamaguti, 1963 (Monogenea: Macrovalvitrematidae) Parasitic on *Orthopristis ruber* (Cuvier) (Osteichthyes: Haemulidae) from the Brazilian Coast. *Revista Brasileira de Parasitología Veterinaria* (Brazilian Journal of Veterinary Parasitology) 2: 111-114
- Mamaev, Y. L. 1976. [The system and phylogeny of monogeneans of the family Diclidophoridae]. In: *Fauna and systematic and phylogenetics of Monogenoidea*. Trudy biologo-Pochvennogo Instituta, Novaya Seriya 35: 57-80. (In Russian).
- Mamaev, Y. L. 1976. [The system and phylogeny of monogeneans of the family Diclidophoridae]. In: *Fauna and systematic and phylogenetics of Monogenoidea*. Trudy biologo-Pochvennogo Instituta, Novaya Seriya 35: 57-80. (In Russian).

- Mollaret, I. BGM. Jamieson and A. D. Adlard et al. Phylogenetic analysis of the Monogenea and their relationships with Digenea and Eucestoda inferred from 28S rDNA sequences. *Mol. Biochim Parasitol.* 90: 433-438.
- Mollaret, I, B. G. M. Jamieson and J-L Justine. 2000. Phylogeny of the Monopisthocotylea and Polyopisthocotylea (Platyhelminthes) inferred from 28S rDNA sequences. *International Journal for Parasitology* 30: 171-185.
- Olson, P. D. and D.T.J. Littlewood. 2002. Phylogenetics of the Monogenea – evidence from a medley of molecules. *International Journal for Parasitology* 32: 233-244
- Payne, R. R. 1987. Two New Monogenea (Macrovalvitrematidae) From Eastern Pacific Ocean Fishes.
- Pearse, A. S. 1949. Observations on Flatworms and Nemerteans Collected at Beaufort, N. C. *Proceedings of the United States National Museum* 100: 25-38.
- Pérez-Ponce de León, G., V. León-Regagnon and B. Mendoza-Garfias. 1997. Análisis Filogenético de la Familia Pterinotrematidae (Platyhelminthes: Cercomeromorpha: Monogenea). *Anales del Instituto de Biología de la Universidad Autónoma de México, Serie de Zoología* 68: 193-205.
- Poulin R. 2002. The evolution of monogenean diversity. *International Journal of parasitology.* 32:245-254.
- Ronquist, F. and J. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inferences under mixed models. *Bioinformatics* 19: 1572-1574.
- Rubec, L. A. and N.O. Dronen. 1994. Revision of the genus *Diclidophora* Kroyer, 1838 (Monogenea: Diclidophoridae), with the proposal of *Macrouridophora* n.g. *Systematic Parasitology* 28: 159-185.
- Sproston, N. G. 1946. A synopsis of the monogenetic trematodes. *Trans. Zool. Soc. London*, XXV, 4: 185-600.
- Suriano, D. M. 1975. Sistemática, biología y microecología de tres Monogenea, Polyopisthocotylea parásitos de las branquias de *Micropogonias opercularis* (Quoy y Gaimard) y *Umbrina canosai* Berg (Pisces, Sciaenidae) del Océano Atlántico Sudoccidental. *Physis. Sección A; Los Océanos y sus organismos* 34: 147-163.
- Wheeler, T. A. and L. A. Chisholm. 1995. Monogenea versus Monogenoidea: the case of stability in nomenclature. *Systematic Parasitology* 30: 159-164.
- Yamaguti, S. 1963. *Systema Helminthum* Volume IV. Monogenea and Aspidocotylea. Interscience, London. 699 pp.

## Chapter IV.

### Reconstruction of Host Phylogeny based on the Parasite Data: notes on coevolutionary events and host specificity

**Abstract:** Monogeneans are among the most host-specific parasites and may be the most host-specific of all fish parasites. Parasite-host specificity is a simple concept encompassing the evolutionary history of associations with different host taxa. Host parasite coevolution has been the subject of numerous studies. Coevolution occurs when two species interact with each other intimately enough and sufficiently long to affect each other's evolution. The reconstruction of the host phylogeny was created with the parasite data and performed using Brooks Parsimony Analysis (BPA) and Bayesian analyses individually. The Macrovalvitrematidae is composed of 14 species parasitizing members of five families of perciform fishes of the suborder Percoidei (Sciaenidae, Haemulidae, Serranidae, Sparidae, Lutjanidae) and one family of pleuronectiform fishes (Paralichthyidae). The results of both the analyses suggest polyphyly for the Superorder Acanthopterygii, Series Percomorpha and proposes the Lutjanidae as a sister group of the Sciaenidae, and the Sparidae as a sister group of the (Lutjanidae, Sciaenidae). The polyphyly represented by the position of a member of the flatfish (Pleuronectiformes) *Citharichthys sordidus* as sister group of the Perciformes, Sparidae, Lutjanidae and Sciaenidae is supported by the oldest ancestor of the macrovalvitrematids. A host-switching event could be suggested with the position of *C. sordidus* associated with the origin of its parasite *Papillopseudotagia hubbsi*. Additionally, there is evidence of co-speciation among the pseudotagid monogeneans and haemulid fishes. Co-occurring species of macrovalvitrematids from two subfamilies occurs in several species of sciaenid and one lutjanid forming communities of macrovalvitremid parasites.

## Introduction

The monogeneans are considered among the most host-specific parasites and may be the most host-specific of all fish parasites (Whittington et al., 2000). Rohde (1982) suggests that Monogenea have the highest degree of host specificity among parasites of marine fishes. The mechanisms responsible for host specificity are one of the main issues in parasitology (Buchmann and Lindestrom, 2002). This concept has been a focus for research in all parasitic groups and has contributed many hypotheses about what mediates or controls the phenomenon (Whittington et al., 2000). Parasite-host specificity is a simple concept incorporating the evolutionary history of associations with different host taxa (Caira et al., 2003). According to Poulin (2002) the monogeneans tend to be host specific, i.e. each monogenean species infects only one or very few host species, and he considered that this is not necessarily the outcome of strict cospeciation between the parasites and their hosts.

Sasal et al., (1999) considered that even if there are no clear causes of specificity, it is possible that specialization is an adaptation of a parasite and that the “generalist vs. specialist strategy” for a parasite consists of expanding or reducing its host range. Ward (1992) listed three factors to explain the evolution of host specificity including: characteristics of the host that necessitate specific adaptations in the parasite; restriction of the parasite’s niche by competition or predation; and specificity as a way of finding mates. Poulin (1992) considered the monogeneans good candidates for investigating the evolution of parasite specialization.

Host parasite coevolution has been the subject of numerous studies for a long time (Desdevises et al. 2002). Some works dedicated to studying host-parasite coevolution are Bychowsky (1961),

Brooks (1979), Hafner and Nadler (1988), Kassen and Beverley-Burton (1988), Klassen (1992) Page (1993, 1994), Boeger and Kritsky (1997). Desdevise, (2002) and others recognize that the “Farenholz’ rule”, (which indicates that parasite phylogeny mirrors host phylogeny) does not seem to be generally true. Coevolution occurs when two species interact with each other intimately enough and sufficiently long enough to affect each other’s evolution (Ronquist, 1997). Ronquist (1997) refers to coevolution as “an exceedingly common phenomenon”. Desdevise (2002) used the term “coevolution” to describe the extent to which the host and parasite phylogenetic trees are congruent. When the trees are perfectly congruent coevolution is the equivalent of cospeciation. According to Poulin (2002) there is also evidence of host switching during coevolutionary history of monogeneans and their hosts, based on studies both at the level of sister species and among more basal branches in the monogenean phylogeny.

In coevolutionary analysis four different types of events need to be considered: duplication, host shifts, sorting events (extinction) and cospeciations (Ronquist, 1997). Ronquist (1997) speculated that duplications occur when parasites speciate independently of their hosts, but remain associated with their ancestral host; host shifts are often considered as being associated with parasite speciation in which one daughter parasite lineage shifts to a new host; sorting events occur when a parasite and host cospeciate, but the parasites become extinct in one of the daughter host lineages; and cospeciations involving simultaneous host and parasite speciation.

The macroalvitrematids have demonstrated preference for the sciaenid fishes, especially the genus *Micropogonias*. Although they show preference for and have higher intensities of infections in the sciaenid fishes, this family has also been reported from other percoid families

and one pleuronectiform species. The relationship established by the macrovalvitrematids among the Sciaenidae and other percoid families has been supported by analyses of other monogenean parasites (Boeger and Kritsky, 2003). This work represents the first reconstruction of a phylogeny of fishes based on their macrovalvitrematid parasites in order to address coevolutionary events associated with the macrovalvitrematids and their hosts.



## Review of Literature

Brooks and McLennan, (1993) stated that “host-parasite associations reflect a long history of reciprocal evolution, during which the ecology of both hosts and parasites are strongly linked”..

Freeland (1983) emphasized that parasites may have effects on the structure and species composition of animal communities and suggested that the phylogenetic backgrounds, body sizes and morphologies, and feeding behaviors of animal species promote parasite-host specificity.

Boeger and Kritsky (1997) used their new phylogeny (Boeger and Kritsky, 1993) to determine coevolutionary relationships at the family level of Monogenea with their hosts. Their analysis suggests that the Monogenea apparently underwent sympatric speciation or dispersal while parasitic on ancestral Gnathostomata, resulting in two primary clades: Polyonchoinea and the Oligonchoinea + Polystomatoinea. Huyse and Volckaert (2002) studied the host-parasite relationship between *Pomatoschistus-Gyrodactylus* with morphometric and molecular analyses. They compared host and parasite phylogeny, examined the ecological and phylogenetic processes involved in this host-parasite system, as well as evaluated phenomena such as coevolution and host-switching.

Whittington (1998) suggested that the number of monogenean species may be at least as high as the number of fish species with a tentative estimate of more than 25,000 species. Sasal and Morand (1998) investigated the relationship between monogeneans and their hosts to answer why some host species have more parasites than others, to understand what factors are related to monogenean species richness and the differences in specificity. Sasal et al. (1999) compared generalists (found on more than one species) vs. specialists (found on only one host species) monogenean parasites of fishes, and found that specialists parasitize larger hosts than generalists. Whittington et al. (2000) developed the concept for a potential role in host-specificity by the anterior

adhesive areas, either the specialized tegument and/or anterior secretions produced by monogeneans for firm attachment during locomotion on host epithelial surfaces.

Desdevises et al. (2000) used DNA sequences from four monogenean species of the genus *Lamellodiscus* and their three fish host species of genus *Pagellus* in order to estimate the molecular divergence and the coevolutionary interactions in this association. Also Desdevises et al. (2002b) studied host-parasite coevolution between Sparidae (Teleostei) fishes and their parasites of the genus *Lamellodiscus* with the reconstruction of molecular phylogenies for both groups.

Bakke et al. (2002) studied the dynamics of host specificity of gyrodactylid monogeneans. Buchmann and Lindstrom (2002) studied the parasite factor associated with recognition and selection of the host, and the host mechanisms responsible for acceptance or rejection of monogeneans. Poulin (2002) examined monogenean diversity in order to determine whether the diversification of monogeneans is driven by some ecological features or by extrinsic factors associated with the host. Morand et al (2002) analyzed patterns of species richness, host specificity, community structure and host-parasite coevolutionary interactions and proposed a hypotheses of evolutionary processes. Bentz et al. (2003) provided a new insight for coevolutionary scenarios between monopisthocotyleans and gnatostomatan hosts.

## Material and Methods

The reconstruction of the host phylogeny was created with the parasite data and performed using Brooks Parsimony Analysis (BPA) (Brooks and McLennan, 1993). A number was assigned to each terminal (extant) taxa and each common ancestor or node of the parasite phylogenetic hypothesis. These numbers were used to create a data matrix listing the binary code for each host indicating the phylogenetic relationships among the 11 species of macroalvitrematids (See Tables III-1 and III-2) based on the hypotheses resulting from parsimony and Bayesian analyses respectively.

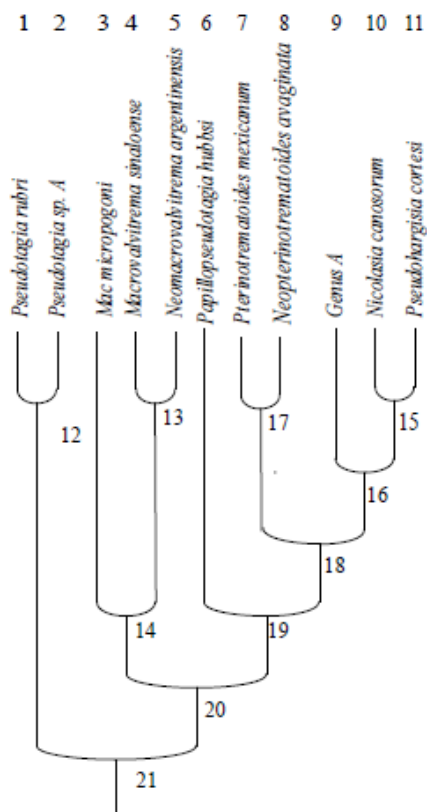
According to the results of the character analysis and supported by the phylogenetic hypotheses resulting from parsimony and Bayesian analyses, the genera *Neomacroalvitrema sinaloense* and *Pseudohargisia cortesi* were reassigned to the genera *Macroalvitrema argentinensis* and *Nicolasia cortesi* respectively. Therefore in this chapter will be referred to those as *M. argentinensis* n. comb. and *N. cortesi* n. comb.

The reconstruction of the characters (based on the macroalvitrematid species and their ancestors) were performed using the program Nona (Goloboff, 1993). The analysis used mult 20 and mult 50, heuristic analysis with 100 replicates (hold max tree= 100; Mult\*100; hold/ 2' Multiple TBR+ TBR multi\*Max). The character examination in the most parsimonious cladograms was made using WinClada. Both optimizations ACCTRAN (fast optimization) and DELTRAN (slow optimization) in WinClada were used to analyze the multiples changes in character states. The hypothesis resulting from each analysis (parsimony and Bayesian) were compared.

The prevalence, mean intensity, mean abundance and the range of the macrovalvitremitid monogeneans collected in the present study from the sciaenid *Micropogonias furnieri*, the haemulid *Pomadasys corvinaeformis* and the sparid *Archosargus rhomboidalis* were calculated to estimate the host specificity of these macrovalvitremitids.

**Table IV-1.** Data matrix of binary codes for species of macrovalvitrematid (from hypothesis resulting from the parsimony analysis) used for the construction of the hypothesis of host phylogeny

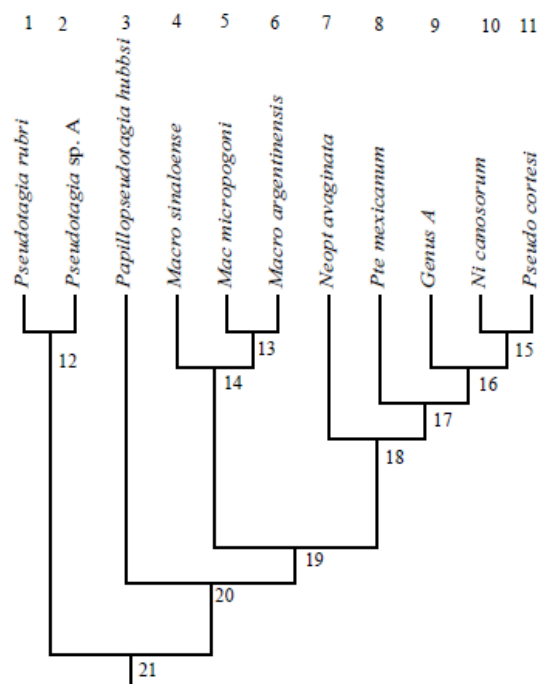
Host Family	Host Genera	Parasite Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Sciaenidae	<i>Micropogonias</i>	3, 4, 5, 7, 8, 11	0	0	1	1	1	0	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1
	<i>Umbrina</i>	4, 7, 10	0	0	0	1	0	0	1	0	0	1	0	0	1	1	1	1	1	1	1	1	1
	<i>Ophioscion</i>	4, 7	0	0	0	1	0	0	1	0	0	0	0	0	1	1	0	0	1	1	1	1	1
Lutjanidae	<i>Lutjanus</i>	4, 7	0	0	0	1	0	0	1	0	0	0	0	0	1	1	0	0	1	1	1	1	1
Sparidae	<i>Archosargus</i>	9	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	1	1	1
Haemulidae	<i>Pomadasys</i>	2	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
	<i>Orthopristis</i>	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
	<i>Haemulon</i>	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Serranidae	<i>Diplectrum</i>	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Paralichthyidae	<i>Citharichthys</i>	6	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1



**Figure IV-1.** Phylogenetic relationship (resulting from the parsimony analysis) for the species of macrovalvitrematid monogeneans numbered for BPA (Brooks Parsimony Analysis) (Brooks and McLennan, 1993). Numbers (12-21) refer to the ancestor of each clade. Each of those numbers refers to the character that appears in Table II-6.

**Table IV-2.** Data matrix of binary codes for species of macrovalvitrematid (from hypothesis resulting from the Bayesian analysis) used for the construction of the hypothesis on host phylogeny

Host Family	Host Genera	Parasite Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Sciaenidae	<i>Micropogonias</i>	4, 5, 6, 7, 8, 11	0	0	0	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1
	<i>Umbrina</i>	5, 8, 10	0	0	0	0	1	0	0	1	0	1	0	0	1	1	1	1	1	1	1	1	1
	<i>Ophioscion</i>	5, 8	0	0	0	0	1	0	0	1	0	0	0	0	1	1	0	0	1	1	1	1	1
Lutjanidae	<i>Lutjanus</i>	5, 8	0	0	0	0	1	0	0	1	0	0	0	0	1	1	0	0	1	1	1	1	1
Sparidae	<i>Archosargus</i>	9	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1
Haemulidae	<i>Pomadasys</i>	2	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
	<i>Orthopristis</i>	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
	<i>Haemulon</i>	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Serranidae	<i>Diplectrum</i>	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Paralichthyidae	<i>Citharichthys</i>	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1



**Figure IV-2.** Phylogenetic relationship (resulting from the Bayesian analysis) for the species of macrovalvitrematid monogeneans numbered for BPA (Brooks Parsimony Analysis) (Brooks and McLennan, 1993). Numbers (12-21) refers to the ancestor of each clade. Each of those numbers refers to the character that appears in Table II-6.

## Results and Discussion

### Hosts

The Macrovalvitrematidae is composed of 14 species parasitizing members of five families of perciform fishes of the suborder Percoidei (Sciaenidae, Haemulidae, Serranidae, Sparidae and Lutjanidae) and one family of pleuronectiform fishes (Paralichthyidae) (Figure III-3). The Percoidei is an immense group containing over 155 families, living in both marine and fresh waters and most have not diverged greatly morphologically (Bond, 1996). Many percoid families are important food fishes including the Lutjanidae (snappers), Serranidae (sea basses and groupers), Sciaenidae (drums and croakers), and Carangidae (jacks and pompanos) among many others.

The Family Sciaenidae has been reported as the most frequent host of the macrovalvitrematids (Figure 3 and 4), being parasitized by six of the nine genera. The Sciaenidae includes about 70 genera and 270 species (Chao, 1978) of primarily marine fishes occurring in the warm coastal and estuarine waters of the Atlantic, Indian and Pacific Oceans (Nelson, 1994; Boeger and Kritsky, 2003). The genus *Micropogonias* is a particularly frequent host with five of the nine genera including *Macrovalvitrema sinaloense*, *Macrovalvitrema argentinensis*, *Macrovalvitrematoides micropogoni*, *Pterinotrematoides mexicanum*, *Neopterinotrematoides avaginata* and *Nicolasia cortesi*. *Nicolasia canosorum* and *Hargisia bairdiella*, infect the gills of other sciaenids *Umbrina coroides* and *Bairdiella chrysoura* respectively.

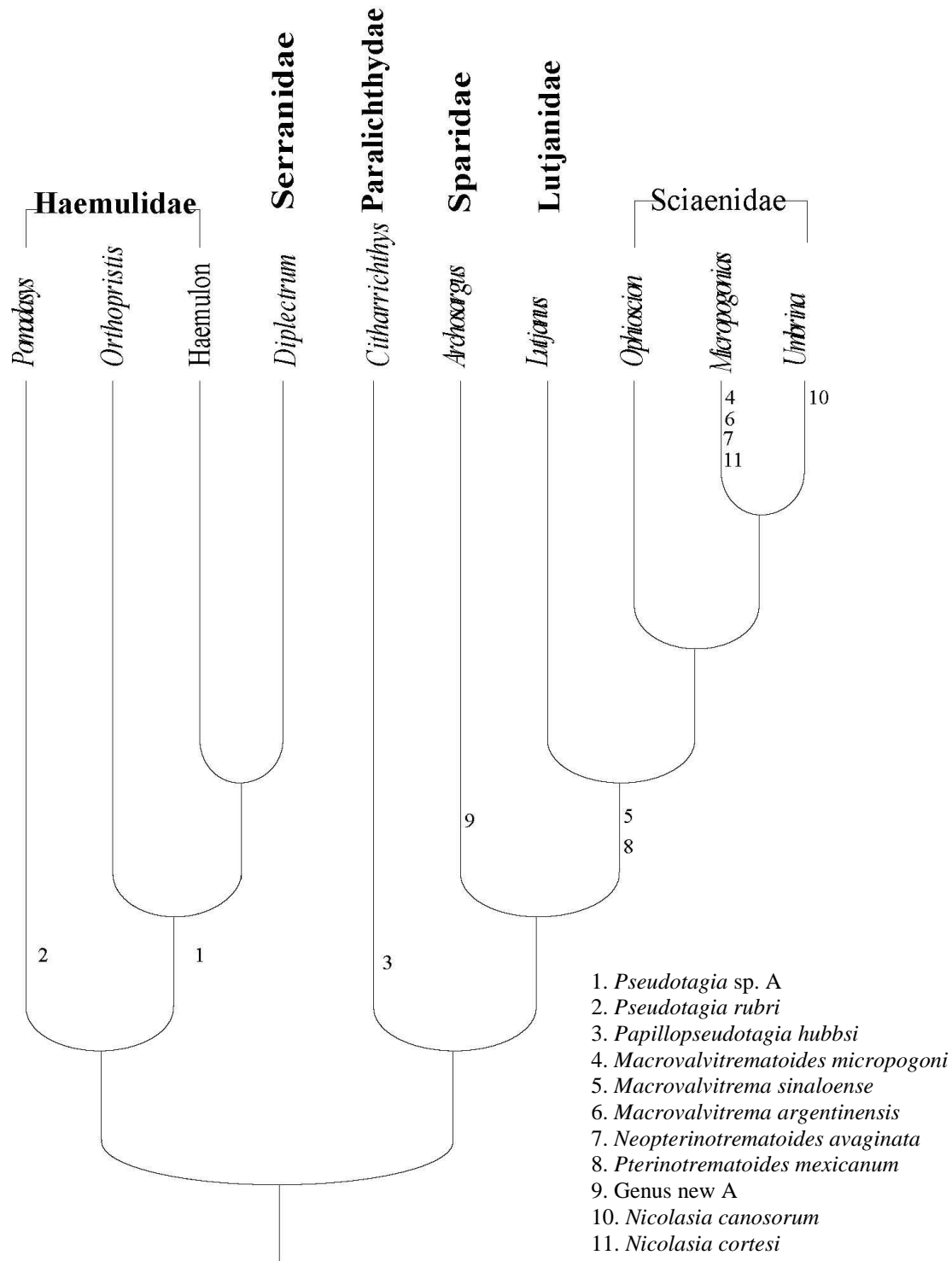
Genus A, besides presenting several autapomorphies, is the only macrovalvitrematid found from the family Sparidae. The monotypic genus *Papillopseudotagia* is the only macrovalvitrematids

reported to infect a flatfish (Family Paralichthyidae). The haemulids and the serranids have been reported as hosts of the four species of *Pseudotagia*.

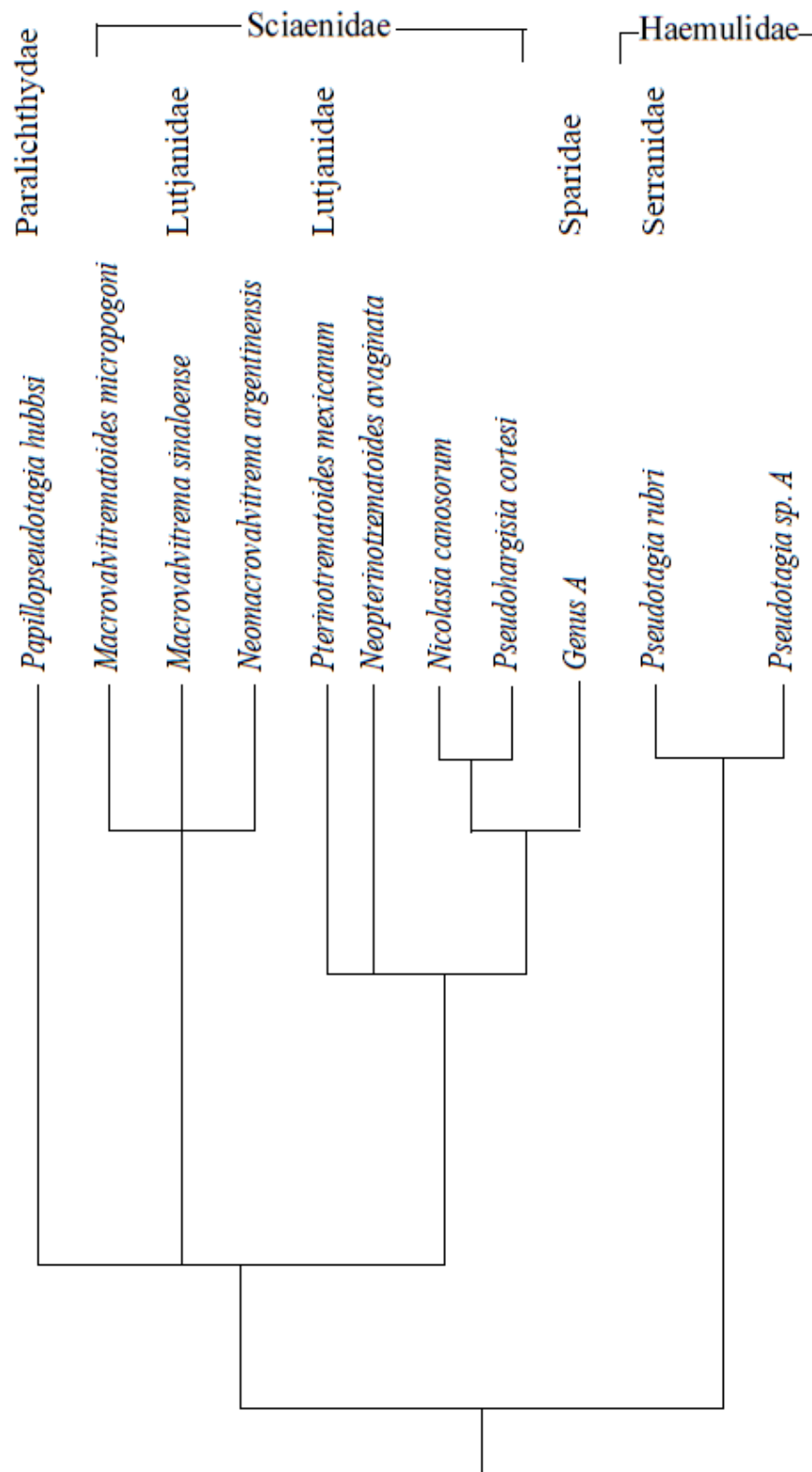
**Table IV-3 Host Families and species of the Macrovalvitrematid Monogeneans**

Host Family	Host species	Macrovalvitrematid Monogenean
Sciaenidae	<i>Micropogonias ectenes</i>	<i>Macrovalvitrema sinaloense</i> <i>Pterinotrematoides mexicanum</i>
	<i>M. megalops</i>	<i>Macrovalvitrema sinaloense</i> <i>Pterinotrematoides mexicanum</i> <i>Nicolasia cortesi</i> n. comb
	<i>M. furnieri</i>	<i>Macrovalvitrema argentinensis</i> n. comb <i>Neopterinotrematoides avaginata</i>
	<i>M. undulatus</i>	<i>Macrovalvitrematoides micropogoni</i>
	<i>Umbrina canosoi</i>	<i>Nicolasia canosorum</i>
	<i>U. roncadore</i>	<i>Macrovalvitrema sinaloense</i> <i>Pterinotrematoides mexicanum</i>
	<i>Ophioscion scierus</i>	<i>Macrovalvitrema sinaloense</i> <i>Pterinotrematoides mexicanum</i>
	<i>Bairdiella chrysoura</i>	<i>Hargisia bairdiella</i>
	<i>Lutjanus argentiventris</i>	<i>Macrovalvitrema sinaloense</i> <i>Pterinotrematoides mexicanum</i>
	<i>Orthopristis chrysopterus</i> <i>O. ruber</i>	<i>Pseudotagia cupida</i> <i>Pseudotagia rubri</i> <i>P. cupida</i>
		<i>Pseudotagia rubri</i>
		<i>Pseudotagia n. sp. A</i>
Serranidae	<i>Paralabrax clathratus</i>	<i>Pseudotagia clathratus</i>
	<i>Diplectrum</i> sp.	<i>Pseudotagia rubri</i>
Sparidae	<i>Archosargus rhomboidalis</i>	Genus n. A
Paralichthyidae	<i>Citharichthys sordidus</i>	<i>Papillopseudotagia hubbsi</i>

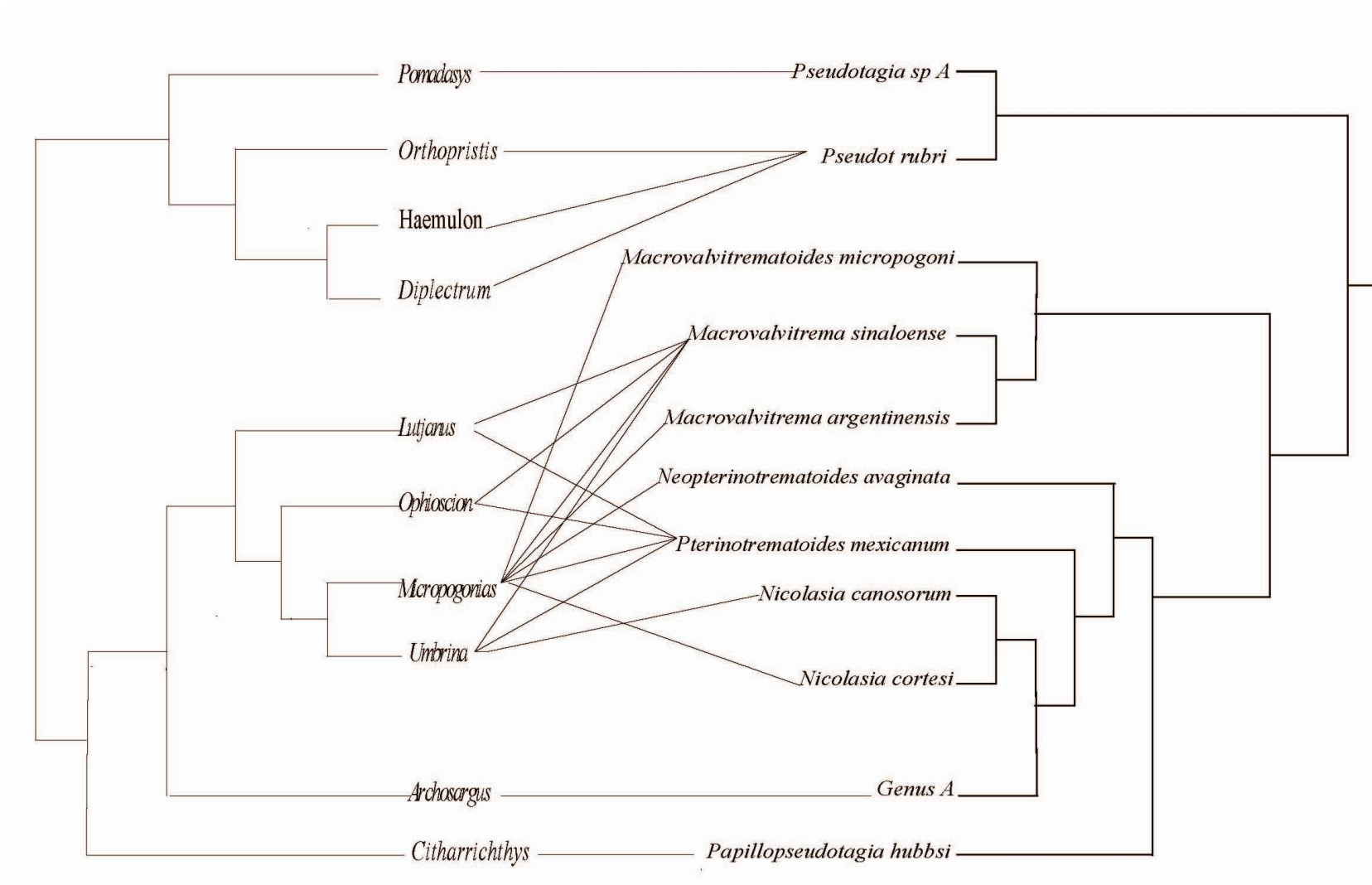




**Figure IV-3.** Phylogenetic Hypothesis for the host genera of members of the family Macrovalvitrematidae Yamaguti, 1963 constructed from the parasite data (of the hypothesis resulting from the parsimony analysis) and performed using Brooks Parsimony Analysis (BPA) (Brooks and McLennan, 1993. Length of tree= 22 steps; consistency index= 95%; and retention index= 97%.



**Figure IV-4.** Relationship between the species of the family Macrovalvitremitidae Yamaguti, 1963 and their host families. Species not represented in the cladogram are *Hargisia bairdiella*, *Pseudotagia cupida* and *P. clathratus* that parasitize the sciaenid *Bairdiella ronchus*, the haemulid *Orthopristis chrysopterus* and the serranid *Paralabrax clathratus* respectively. The hosts of these three species support the relationship established by the other species. (The diagram used is the consensus tree resulting from the Bayesian analysis)



**Figure IV-5.** Macrovalvitrematid-Host Phylogenies: Phylogenetic hypothesis of the Family Macrovalvitrematidae Yamaguti, 1963 resulting from the parsimony analysis (left), vs Phylogenetic hypothesis of the host of macrovalvitrematid based on the parasite data (using BPA) (right). The lines between the two trees refers to the presence of the parasites in certain host genus.

### Reconstruction of host phylogeny

The phylogenetic hypothesis for the macroalvitrematid host genera was constructed from the parasite data from hypotheses obtained from both parsimony and Bayesian methods and performed with the Brooks parsimony analysis (Brooks and McLennan, 1993). The phylogenetic analyses with both methods resulted in the same host phylogenetic cladogram with a length of 22 steps, a consistency index of 95% and retention index of 97%.

This phylogenetic topology suggests polyphyly for the Superorder Acanthopterygii, Series Percomorpha, and the serranid conferring paraphyletic status to the Haemulidae. The hypothesis also proposes the Lutjanidae as a sister group of the Sciaenidae, and the Sparidae as the sister group of the (Lutjanidae, Sciaenidae). The evolution of these associations suggested by this hypothesis will be discussed below: (Host Fish Genera= Taxa; Macroalvitrematid monogenean parasites= Characters); Numbers 12-21 refer to the clade (common ancestor) of the phylogenetic hypothesis resulted from the parsimony analysis of the Family Macroalvitrematidae (Figure 32). The discussion will begin with the information obtained from the stems (established by the extant macroalvitrematid genera) followed by the information of the nodes (ancestors).

- Fishes of the Families Haemulidae and Serranidae are indicated as evolved from a common ancestor based in the synapomorphies established by the presence of species of the macroalvitrematid genus *Pseudotagia* (Figure III-1 and III-2, number 12, Figure III-5). *Pseudotagia rubri* represents a synapomorphy for the haemulid genera *Orthopristis* and *Haemulon* and for the serranid *Diplectrum*, while *Pseudotagia* sp. A represents an autapomorphy for the haemulid *Pomadasys* (Figure III-3). The other two species of the genus *Pseudotagia* (not included in the analysis because of insufficient information) also

support this host relationship. *Pseudotagia cupida* has been reported from the haemulid *Orthopristis chrysopterus* while *P. clathratus* is from the serranid *Paralabrax clathratus*

- A close relationship of the family Sciaenidae with the Lutjanidae is suggested by the hypothesis with seven synapomorphies supporting it. These synapomorphies include the presence of the macrovalvitrematid species *Macrovalvitrema sinaloense* and *Pterinotrematoides mexicanum* and the presence of their respective ancestor (Figure III-5).
- The presence of the macrovalvitrematids *Macrovalvitrematoides micropogoni*, *Macrovalvitrema argentinensis*, *Neopteriotrematoides avaginata* and *Nicolasia cortesi* are suggested as autapomorphies for the sciaenid host *Micropogonias*, while the genus *Nicolasia* establishes an autapomorphy for the sciaenid host *Umbrina*. The hypothesis suggests that these two sciaenid genera are more related than the sciaenid genus *Ophioscion* supporting it with one synapomorphy: the presence of the common ancestor of (*Nicolasia canosorum*, *N. cortesi*). Chao (1978) grouped these two genera closer than the genus *Ophioscion* based on the external morphology.
- The macrovalvitrematids Genus A and *Papillopseudotagia* represent an autapomorphy for the sparid *Archosargus rhomboidalis* and the paralichthid *Citharichthys sordidus* respectively.
- The common ancestor of the macrovalvitrematids (Genus A, (*Nicolasia canosorum*, *N. cortesi*)) suggests a homoplasy in the cladogram, being present in the sparid *Archosargus rhomboidalis* and in the sciaenids *Umbrina* and *Micropogonias*.
- The position of the Sparidae (represented by a single species, *Archosargus rhomboidalis*) as sister group of (Sciaenidae and Lutjanidae) is supported by the most recent common

ancestor of the macrovalvitrematids *Pterinotrematoides*, *Neopterinotrematoides*, *Nicolasia* and Genus A.

- The position of the Paralichthyidae (also represented by a single species, *Citharichthys sordidus*) is supported by the two (or three using the Bayesian hypothesis of the parasite data) oldest ancestors of the macrovalvitrematid monogeneans.
- The basal position of the Haemulidae (and the Serranidae conferring it a paraphyletic status) is supported only by the oldest ancestor of macrovalvitrematids.

### Co-evolutionary Comments and Events

1. The polyphyly represented by the position of a member of the flatfish (Pleuronectiformes) *Citharichthys sordidus* as sister group of the perciform Families Sparidae, Lutjanidae and Sciaenidae is supported by the oldest ancestors of the macrovalvitrematids. Johnson (1993) suggests that the perciformes, which is the largest and most diverse of the percomorph orders, are probably polyphyletic and that the relationship of pleuronectiforms to other percomorphs remains obscure. Within percoids the major advances have been definition of monophyletic families and the reordering of genera, but relationships above the family level have remained difficult to define and no complete or partial phylogeny has been proposed for the percoidei. In addition there is no evidence that it is a monophyletic group (Johnson, 1993). He (Johnson, 1993) comments that “the same can be said for the order Perciformes, for which limits and monophyly of many suborders have been established, but their interrelationships remain obscure and there is no evidence of their monophyly”.

- If monophyly of the host families of macrovalvitrematids is assumed, however, the position of *C. sordidus* suggests a host-switching event associated with the origin of *Papillopseudotagia hubbsi* on *C. sordidus*. This could explain the autapomorphies presented by this species (e.g the separation of the lateral sclerites in one side of the ventral valve, the reduction of the plate, their limited orientation along one lateral sclerite in all their clamps and the presence of papillae in the ventral valve of clamps among others).
2. This cladogram, based on the information of the macrovalvitrematid data, establishes a relationship among the perciform Families Haemulidae, Serranidae, Sparidae, Lutjanidae and Sciaenidae. This relationship has been previously supported by Chao (1978), who stated that Sciaenidae is most similar to Lutjanidae, Pomadasyidae (= Haemulidae), and Sparidae among other closely related perciform families based on the morphology of otholiths described by Schmidt (1968) (Boeger and Kritsky, 2003). Boeger and Kritsky (2003) established that a phylogenetic relationship exists between these perciform families and that it is supported by the extant distribution of *Euryhaliotrema* spp. (Polyonchoinean monogenea). According to the parasite data (of *Euryhaliotrema* spp.) used by Boeger and Kritsky (2003) Sparidae is the sister group of Sciaenidae, while both Lutjanidae and Haemulidae comprise distinct basal lineages.
- The only lutjanid species reported as a host of the Macrovalvitrematidae is endemic in the eastern Pacific Ocean from southern California to Perú and can be found in fresh, brackish and salt waters. The family Lutjanidae was not captured

and thus not examined in the present study, but previous studies of metazoan parasites of snappers of Puerto Rico by Bosques-Rodríguez (2004) did not report the presence of any macrovalvitrematids. Macrovalvitrematids also have not been reported from the lutjanids of the south and north Atlantic.

3. Sasaki (1989) related the Sciaenidae with the Haemulidae by the common presence of chin pores, and with both Haemulidae and Lutjanidae because of the similarity in the sulcus pattern on the saggita in their otoliths. But, in conclusion, Sasaki (1989) established that the Sciaenidae is a group belonging to the Percoidei, representing an early offshoot in percoid evolution, and that neither the evidence previously presented in the literature, nor characters examined in his study enabled the identification of a sciaenid sister group. Johnson (1993) recommended that families Sciaenidae and Polynemidae should be included in a superfamily Polynemoidea based on the similarity of the larval forms as evidence to support this hypothesis that the Sciaenidae and Polynemidae are sister groups.
- Although the present study does not signify the real phylogenetic relationship among the perciform fishes, with the exception of *Citharrichthys*, the host phylogeny created with macrovalvitrematid data clearly support the relationship established by other monogenean parasite (*Euryhaliotrema*) by Boeger and Krtisky (2003).



- The present analysis places the Sciaenidae in a terminal clade with the Lutjanidae (only one species, *Lutjanus argentiventris*) as their sister group. This close relationship between sciaenids with the lutjanids is attributed by the presence of *Macrovalvitrema sinaloense* and *Pterinotrematoides mexicanum* in the sciaenids *Micropogonias ectenes*, *M. megalops*, *Umbrina roncadore*, *Ophioscion sciurus* and in the lutjanid *L. argentiventris*, an endemic lutjanid from the eastern Pacific, as reported by Bravo-Hollis (1982).
- The cladogram suggests the Sparidae as the sister group of (Sciaenidae, Lutjanidae) based on the presence of the macrovalvitrematid Genus A on *Archosargus rhomboidalis*. This macrovalvitrematid has acquired several highly distinctive autapomorphies such as an increase of the width of the anterior end, the enlargement of the oral suckers with slight sclerotization on the borders, the absence of a pharynx and several characters related to the clamps.
- Finally the hypothesis places the Haemulidae in the basal clade with the Serranidae conferring a paraphyletic status to it. The basal position of the Haemulidae is congruent with the basal position of the genus *Pseudotagia* obtained from the phylogenetic analysis of the family Macrovalvitrematidae using parsimony and Bayesian methods (Chapter II Figures 32 and 33 respectively) suggesting cospeciation of the pseudotagid macrovalvitrematid with the haemulid fishes.
- The specimens of *Pseudotagia rubri* reported from the serranid fish *Diplectrum* sp. may be erroneous identifications or subjected to misinterpretation. Originally the specimens from *Diplectrum* sp. were reported by Kohn et.al., (1992) as

*Pseudotagia cupida* Hargis, (1956) originally described from *Orthopristis chrysopteron* from the Gulf of Mexico. Later Luque et al. (1993) erected *Pseudotagia rubri* from *Orthopristis ruber* and placed Kohn's (1992) specimens from *Diplectrum* sp. in this new species based on the description made by Kohn et al (1992). No one compared their material to the type material deposited by Hargis (1956) as this material has been lost. Thus it is possible that the material from *Diplectrum* sp. could be another new species of *Pseudotagia* and may represent a host switching event.

4. Although the macroalvitrematids have demonstrated preferences for the percoid host families Sciaenidae and Haemulidae, this monogenean family with so few species has demonstrated a relatively wide host range including five host families. According to Rohde (1982) parasites infecting single host taxon or related taxa are said to exhibit phylogenetic host specificity. This represents the results of co-speciation of the host and the parasites. According to Sasal et al. (1999) the host-parasite associations may reflect a long history of co-evolution, during which the ecology of the parasite is strongly linked with that of the host. But even parasites with a wide host range always show certain host preferences, but these preferences are usually determined by the ecological requirements of the host (Rohde, 1982). He refers to these parasites as ones exhibiting ecological host specificity.
- All the hosts reported for the macroalvitrematids inhabit brackish waters and muddy or sandy areas suggesting that macroalvitrematids from Sparidae,

Lutjanidae and Paralichthyidae have a certain degree of ecological host specificity. But the preference of the genera *Macrovalvitrema sinaloense* and *Pterinotrematoides mexicanum* for the Sciaenidae; *Macrovalvitrema argentinensis*, *Neopterinotrematoides avaginata*, *Hargisia bairdiella* and *Macrovalvitrematoides micropogoni* for the sciaenid of the genus *Micropogonias*; and the genus *Pseudotagia* for the Haemulidae are a result of phylogenetic host specificity resulting from co-speciation.

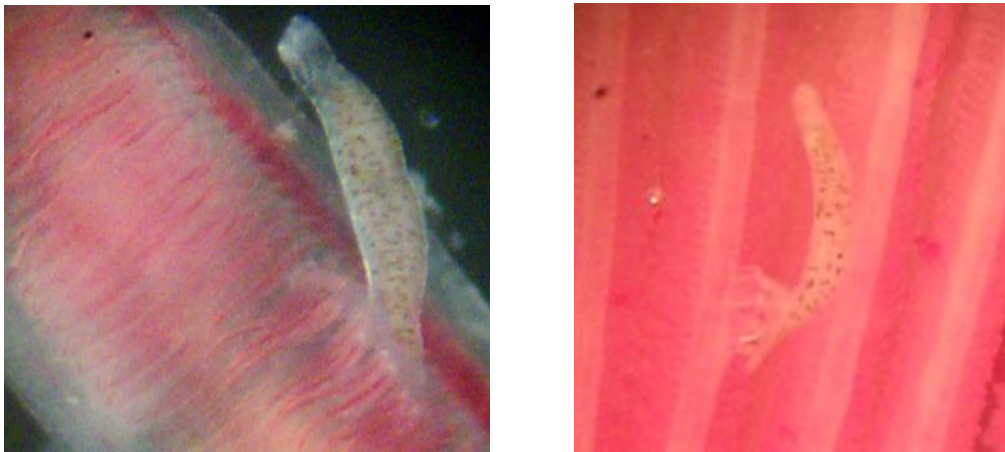
### **Co-occurrence of Macrovalvitrematinae and Pterinotrematoidinae**

Several hosts have two macrovalvitremids species that occur together on the same individual host (co-occur). *Macrovalvitrema sinaloense* and *Pterinotrematoides mexicanum* are found together on the same individuals of *Micropogonias ectenes*, *M. megalops*, *Ophioscion sciurus*, *Umbrina roncadore* and *Lutjanus argentiventris* representing two populations that exist as a community on the gills of these hosts. This co-occurrence establishes a relationship between a macrovalvitrematid of the subfamily Macrovalvitrematinae (slight strangulation of the clamps) and a macrovalvitrematid of the subfamily Pterinotrematoidinae (prominent strangulation at midline level).

This relationship (Macrovalvitrematinae- Pterinotrematoidinae) was also observed in the co-occurring community of macrovalvitrematids observed in the gills of *M. furnieri* from Argentina by Suriano (1975) and also from the same host collected in the present study. *Macrovalvitrema argentinensis* and *Neopterinotrematoides avaginata* were found on the same individual host in 15 of the 35 specimens (43%) of *Micropogonias furnieri* collected from Mayagüez, Puerto Rico.

This co-occurrence could be suggested as the results of the duplication or the independent parasite speciation in which the parasite has speciated but remains in the ancestral host resulting in two related species (resulting from a common ancestor) in the same host. Having the co-occurrence in various sciaenid hosts as well in the lutjanid host (*Lutjanus argentiventris*) in the Pacific coast of Mexico and in the Gulf of California indicates that both have coevolved together with the host.

The macrovalvitrematinae species are larger than the Pterinotrematoidinae species and during the examination of the gills of *M. furnieri*, the individuals of *Macrovalvitrema argentinensis* (Macrovalvitrematinae) were observed most commonly on the midline of the gill filaments while the individuals of *Pterinotrematoides avaginata* (Pterinotrematoidinae) are most commonly found with the clamps attached to various lamellae (Figure III-6).



**FigureIV- 6** Attachment of the opisthaptor to the gills of the sciaenid *Micropogonias furnieri*. **A.** *Neopteriotrematoides avaginata* with their clamps attached to several gill lamellae; **B.** *Macrovalvitrema argentinensis* (with large clamps) attached to the border of the gill filament .

### Notes on Host Specificity

According to Lymberg (1989) and Sasal et al. (1999) host specificity takes into account epidemiological indices, such as the percentage of infected hosts (prevalence) or the intensity of

infection. Table III-4 presents the results of these indices obtained from the examination of the sciaenid *Micropogonias*, the haemulid *Pomadasys* and the sparid *Archosargus* in the present study. These results show higher prevalences in the sciaenids of the genus *Micropogonias* (67.5% and 65%) and the haemulid *Pomadasys* (72%) than in the sparid *Archosargus* (54%).

**Table IV-4.** Values of Prevalence, Mean Intensity, Abundance, Range, Number of host infected and total number of parasites of the species of Macrovalvitrematidae reported from Puerto Rico

Macrovalvitrematid Monogenean	Number of Parasites	Infected Hosts	Prevalence %	Mean Intensity	Abundance	Range
<i>Macrovalvitrema argentinensis</i> *	41	26	65	1.6	1.025	1-7
<i>Neopteriotrematoides avaginata</i> *	74	27	67.5	2.7	1.85	1-10
<i>Pseudotagia</i> new sp. A <sup>†</sup>	49	13	72	3.8	2.72	1-10
Genus new A <sup>‡</sup>	25	13	54	1.9	1.0	1-4

\* Recorded from 40 specimens of the sciaenid *Micropogonias furnieri*; † from 18 specimens of the haemulid *Pomadasys corvinaeformis* and ‡ from 24 specimens of the sparid *Archosargus rhomboidalis*; .

The highest prevalence (with 72%) and the mean intensity value (of 3.8) of the new species of *Pseudotagia* could indicate that this genus is the most host specific of the genera of the family Macrovalvitrematidae found in Puerto Rico, supporting the suggestion of co-speciation between the *pseudotagid* genus and the haemulid hosts as suggested by the host phylogeny reconstructed with the parasite data. It is important to note that the monogenean community of the haemulid *Pomadasys corvinaeformis* included both this new species of *Pseudotagia* together with two species of the Diclidophoridae (*Eurysorchis* and *Choricotyle*) with the pseudotagid macrovalvitrematid the dominant (more numerous) species.

## Conclusions

The phylogenetic tree constructed from the parasite hypotheses suggests a close relationship between the five perciform families, Sciaenidae, Lutjanidae, Sparidae, Haemulidae and Serranidae. The position of the haemulid fishes is congruent with the basal position of the genus *Pseudotagia* suggesting that they have undergone co-speciation. The macrovalvitrematids prefer the sciaenids and the haemulids. The tree also suggests that the presence of *Papillopseudotagia hubbsi* in the paralichthid *Citharichthys* may be a result of host switching. The co-occurrence of *Macrovalvitrema sinaloense* and *Pterinotrematiodes mexicanum* on several sciaenid genera and in the lutjanid *Lutjanus argentiventris* could be the result of duplication. The presence of the macrovalvitrematids with strangulation in their clamps (co-occurring) in the sciaenid fishes could be the result of co-speciation. The numerous autapomorphies present in Genus A could be the result of adaptation to a Sparidae host. The prevalence and intensity values from the present study support the results of the host phylogeny indicating a close relationship of the pseudotagids and the family Haemulidae. The wide range of hosts of macrovalvitrematids and the common habitat of the hosts could be considered as ecologically host specific. Thus the family Macrovalvitrematidae is a useful group for the study of the mechanisms of speciation.

### Literature Cited

- Boeger, W. A. and D. C. Kritsky. 1993. Phylogeny and a revised classification of the Monogenoidea Bychowsky, 1937 (Platyhelminthes). *Systematic Parasitology* 26: 1-32.
- Boeger, W. A. and D. C. Kritsky. 1997. Coevolution of the Monogenoidea (Platyhelminthes) Based on a Revised Hypothesis of Parasite Phylogeny. *International Journal for Parasitology* 27: 1495—1511.
- Bravo-Hollis, M. 1982. Helminths de peces del pacifico mexicano XXXIX. Dos subfamilias nuevas de Monogéneos de la familia Macrovalvitrematidae Yamaguti, 1963. *Anales del Instituto de Biología Universidad Nacional Autónoma de México* 52: 27-38.
- Boeger, W. A. And D. C. Kritsky. 2003. Parasites, fossils and geologic history: Historical biogeography of the South American freshwater croakers, *Plagioscion* spp. (Teleostei, Sciaenidae). *The Norwegian Academy of Science and Letters. Zoologica Scripta* 32: 3-11.
- Bond, C. E. 1996. *Biology of Fishes*. 2<sup>nd</sup> edition. Saunders College Publishing. 750pp.
- Brooks, D. R. 1989. The phylogeny of the cercomeria (Platyhelminthes: Rhabdocoela) and general evolutionary principles. *Journal of Parasitology* 75: 606-616.
- Brooks, D. R. and D. A. McLennan. 1993. *Parascript Parasites and the language of evolution*. Smithsonian Institution. 429pp.
- Brooks, D. R., R. T. O'Grady & D. R. Glenn. 1985. The phylogeny of the cercomeria Brooks, 1982 (Platyhelminthes). *Proceedings of the Helminthological Society of Washington*, 52: 1-20.
- Buchman, K. and T. Lindstrom. 2002. Interactions between monogenean parasites and their fish hosts. *International Journal for Parasitology* 32: 309-319.
- Caballero y C. y Bravo-Hollis, 1955. Trematodos de peces marinos de aguas mexicanas del Océano Pácifico. VIII. Descripción de tres nuevos generos de trematodos monogéneos. *Anales del Instituto de Biología* 16: 89-115.
- Chao, L. 1978. NOAA Technical Report NMFS Circular 415. A Basis for classifying Western Atlantic Sciaenidae (Teleostei: Perciformes). US Department of Commerce National Oceanic and Atmospheric Administration National Marine Fisheries Service.
- Euzet, L. and L. Combes 1980. Les problems de l espece chez les animaux parasites. *Memoires de la Societe zoologique fracaise* 40: 239-285.
- Freeland, v. 1983. Parasites and the coexistence of animal host species. *American Naturalist* 121:

223-236.

- Hargis, W. J. Jr. 1956. Monogenetic Trematodes of Gulf of Mexico Fishes. Part VIII. The Superfamily Diclidophoroidea Price, 1936. (Continued). Helminthological Society 23: 5-13.
- Lymberg, A. J. 1989. Host Specificity, host range and host preference. Parasitology Today 5: 298.
- Johnson, D. 1993. Percomorph phylogeny: progress and problems. Bulletin of Marine Sciences 52: 3-28.
- Klassen G. J. and M. Beverly-Burton. 1988. North American fresh water ancyrocephalids (Monogenea) with articulating haptor bars: host-parasite coevolution. Systematic Zoology. 37: 179-189.
- Luque, J. L., J. F. R. Amato and R. M. Takemoto. 1993. A New Species of *Pseudotagia* Yamaguti, 1963 (Monogenea: Macrovalvitrematidae) Parasitic on *Orthopristis ruber* (Cuvier) (Osteichthyes: Haemulidae) from the Brazilian Coast. Revista Brasileira De Parasitologia Veterinaria (Brazilian Journal of Veterinary Parasitology) 2: 111-114
- Nelson, J. S. 1994. Fishes of the World, 3<sup>rd</sup> Ed. New York: John Wiley & Sons.
- Page, R. D. M. 1993. Genes, organisms and areas: the problem of multiple lineages. Systematic Biology 42: 77-84.
- Page, R. D. M. 1994. Maps between trees and cladistic analysis of historical association among genes, organisms and areas. Systematic Parasitology. 43: 58-77.
- Payne, R. R. 1987. Two New Monogenea (Macrovalvitrematidae) From Eastern Pacific Ocean Fishes.
- Pearse, A. S. 1949. Observations on Flatworms and Nemerteans Collected at Beaufort, N. C. Proceedings of the United States National Museum 100: 25-38.
- Ronquist, F. 1998. Phylogenetic approaches in coevolution and biogeography. Zoologica Scripta 26: 313-322.
- Sasal, P., S. Trouve, C. muller-Graff and S. Morand. 1999. Specificity and host predictability: a comparative analysis among monogeneans parasites of fish. Journal of animal Ecology 68: 437-444.
- Sasaki, K. 1989. Phylogeny of the family Sciaenidae, with notes on its zoogeography (Teleostei, Perciformes). Memoirs of the Faculty of fisheries Hokkaido University 36: 1-137.
- Schmidh, W. 1968. Vergleichend morphologischche Studie uber die otholithen mariner Knochenfishe. Archiv fur Fishereiwissenschaft 19: 1-96.



- Suriano, D. M. 1975. Sistemática, biología y microecología de tres Monogenea, Polyopisthocotylea parásitos de las branquias de *Micropogonias opercularis* (Quoy y Gaimard) y *Umbrina canosai* Berg (Pisces, Sciaenidae) del Océano Atlántico Sudoccidental. Physis. Sección A; Los Océanos y sus organismos 34: 147-163.
- Ward, S. A, 1992. Assessing functional explanations of host specificity. American Naturalist 139: 883-891.
- Yamaguti, S. 1963. Systema Helminthum Volume IV. Monogenea and Aspidocotylea. Interscience, London. 699 pp.

### Final Conclusions

This study provides new information for the family Macrovalvitrematidae Yamaguti, 1963.

- Seine net collections yielded four species of macrovalvitrematid monogeneans from the west and northeast coasts of Puerto Rico. Their adult morphology as well as their larval development) was studied and described, representing the first reports of macrovalvitrematids from Puerto Rico and for the Caribbean for three of the species.
- One new genus and species; one new species; two new locality records and one new host record to the family.
- The study proposed two new combinations *Macrovalvitrema argentinensis* (Suriano, 1975) and *Nicolasia cortesi* (Payne, 1987) based on the character analysis and supported by the phylogenetic analysis.
- As a result of these new findings, performance of the character analysis and with the supporting evidence of the phylogenetic analyses, the family is now composed of nine genera and 14 species parasitizing the gills of fishes of five perciform and one pleuronectiform family.
- The phylogenetic analyses made with 42 characters using parsimony and Bayesian methods suggested the monophyly of the Macrovalvitrematidae with high support values and four synapomorphies indicating that Macrovalvitrematidae should be recognized as valid family.
  - The genus *Pseudotagia* is placed in a basal position using both parsimony and Bayesian methods. The position of *Papillopseudotagia* is controversial, being located differently in each of these cladograms.

- It also is suggested that the Subfamily Diclidophorinae is its sister group thus granting paraphyletic status to the Diclidophoridae.
- The first revised classification based on phylogenetic results included in this study recognize the subfamilies previously erected (Macrovalvitrematinae and Pterinotrematoidinae) and proposed two more new subfamilies to place the genera *Papillopseudotagia* and *Pseudotagia* respectively. The macrovalvitrematid genera are distributed among these subfamilies as following:

Subfamily Macrovalvitrematinae:

Genera *Macrovalvitrema* and *Macrovalvitrematoides*;

Subfamily Pterinotrematoidinae:

Genera *Pterinotrematoides*, *Neopterinotrematoides*, *Nicolasia*, Genus A and *Hargisia* (not examined, but is preserved because it was previously included)

Subfamily new A: Genus *Pseudotagia*

Subfamily new B: Genus *Papillopseudotagia*

- The host phylogeny constructed with the parasite data suggests a close relationship among the perciform families Sciaenidae, Lutjanidae, Sparidae, Haemulidae and Serranidae. This results suggests three of the four possible coevolutionary events in host-parasite associations (host switching, cospeciation and duplication)
  - › The polytomy represented by the position of the pleuronectiform fish *Citharrichtys sordidus* as sister taxa of the Macrovalvitrematidae probably resulted from the host switching of *Papillopseudotagia hubbsi*.

- › The congruence in the basal position of the perciform family Haemulidae and the macroalvitrematids of the genus *Pseudotagia* suggests co-speciation.
  - The strong relationship between these host family and macroalvitrematid genus is also supported by high prevalence and mean intensity values of the new species of *Pseudotagia* (*Pseudotagia* new sp. A) on the sparid *Pomadasys corvinaeformis*. also
- › The co-occurrence of one species of the subfamily Macroalvitrematinae with one species of the subfamily Pterinotrematoidinae could be suggested as the results of duplication or independent parasite speciation.

### Recommendations

The scope of family hosts related to brackish water need to be enlarged to determine if there are more families infected by macroalvitrematids, and, particularly, to examine more genera and species of the families Sparidae and Paralichthyidae from different localities.

The fishes *Bairdiella chrysoura*, *Micropogonias undulatus* and *Orthopristis chrysopterus* need to be re-examined to collect the macroalvitrematids reported from them (*Hargisia bairdiella*, *Macroalvitrematoide*

*s micropogoni* and *Pseudotagia cupida*), so that a neotype may be designated to replace the lost type material. Also the serranid fishes of the genera *Paralabrax* and *Diplectrum* need further examination. Although the relationship between the genus *Pseudotagia* and members of the fish family Haemulidae is well supported with the data for the host phylogeny and parasite phylogeny, it is still necessary to reevaluate the species *Pseudotagia cupida* and to compare it with *Pseudotagia rubri* and the specimens reported by Kohn (2002) from *Haemulon sciurus*. In addition a more thorough examination of the parasite community of haemulids found in brackish waters such as the genera *Orthopristis* and *Pomadasys*, and the ones more associated with the coral reefs such as *Haemulon* need to be performed. A molecular analysis of the members of the subfamily Macroalvitrematinae could establish whether *Macroalvitrema sinaloense*, *M. argentinensis* and *Macroalvitrematoides micropogoni* are really three different species and two different genera, now based on morphology.

The Macroalvitrematidae is still a small family with most of the genera being monotypic indicating that there are probably more species remaining to be discovered. With these

discoveries a more complete host phylogeny could be constructed and a better understanding of the coevolution of hosts and parasites could be elucidated.

**Appendix I-A.** Material examined and data collected from the fishes of the families Sciaenidae, Haemulidae and Sparidae from the west coast (Caribbean) and the north coast (Atlantic) of Puerto Rico

Host family	Host species	Locality	Collection Date	Examination Date	Species richness	Parasite Taxa	Parasite Species	I or A	Slides #
Sciaenidae	<i>Bairdiella ronchus</i>	Espinal, Aguada	Aug/20/08	Aug/22/08	0	-----	-----	----	---
Sciaenidae	<i>B. ronchus</i>	Espinal, Aguada	Aug/20/08	Aug/22/08	1	Digenea	Not identified	1	
Sciaenidae	<i>B. ronchus</i>	Boquilla, Mayagüez	Mar/31/09	Apr/14/09	0	-----	-----	----	----
Sciaenidae	<i>B. ronchus</i>	Boquilla, Mayagüez	Mar/31/09	May/7/09	2	Monogenea: Diclidophoridae Polyonchoinea	<i>Pedocotyle</i> sp. <i>Rhamnocercus</i> sp	2 1	88, V I-C1 V I-C1
Sciaenidae	<i>B. ronchus</i>	Boquilla, Mayagüez	Mar/31/09	Jan/26/10	1	Monogenea: Polyonchoinea	<i>Rhamnocercus</i> sp	10	VI-D1
Sciaenidae	<i>B. ronchus</i>	Boquilla, Mayagüez	Mar/31/09	Feb/2/10	1	Monogenea: Polyonchoinea	<i>Rhamnocercus</i> sp	16	V I-D2, D3
Sciaenidae	<i>B. ronchus</i>	Guanajibo, Mayagüez	Jan/22/11	Jan/22/11	1	Monogenea: Polyonchoinea	<i>Rhamnocercus</i> sp	9	V I-D4
Sciaenidae	<i>B. ronchus</i>	Guanajibo, Mayagüez	Jan/22/11	Jan/22/11	1	Monogenea: Polyonchoinea	<i>Rhamnocercus</i> sp	8	V I-D4
Sciaenidae	<i>B. ronchus</i>	Guanajibo, Mayagüez	Jan/22/11	Jan/22/11	2	Monogenea: Diclidophoridae Polyonchoinea	<i>Pedocotyle</i> sp. <i>Rhamnocercus</i> sp	1 10	97 V I-D4
Sciaenidae	<i>Cynoscion jamaicensis</i>	Guanajibo, Mayagüez	Jan/17/09	Jul/12/10	0	-----	-----	----	----
Sciaenidae	<i>C. jamaicensis</i>	Guanajibo, Mayagüez	Jan/17/09	Jul/12/10	0	-----	-----	----	----
Sciaenidae	<i>C. jamaicensis</i>	Boquilla, Mayagüez	Mar/16/09	Jul/27/10	0	-----	-----	----	----
Sciaenidae	<i>Larimus breviceps</i>	Espinal, Aguada	Aug/16/08	Aug/18/08	0	-----	-----	----	----
Sciaenidae	<i>L. breviceps</i>	Guanajibo, Mayagüez	Jan/17/09	Jun/12/10	0	-----	-----	----	----
Sciaenidae	<i>L. breviceps</i>	Guanajibo, Mayagüez	Jan/17/09	Jun/12/10	0	-----	-----	----	----

Host family	Host species	Locality	Collection Date	Examination Date	Species richness	Parasite Taxa	Parasite Species	I or A	Slides #
Sciaenidae	<i>L. breviceps</i>	Guanajibo, Mayagüez	Feb/4/09	Feb/10/09	0	-----	-----	----	----
Sciaenidae	<i>L. breviceps</i>	Guanajibo, Mayagüez	Jan/22/11	Jan/22/11	0	-----	-----	----	----
Sciaenidae	<i>Menticirrhus americanus</i>	Espinal, Aguada	Aug/16/08	Aug/18/08	0	-----	-----	----	----
Sciaenidae	<i>M. americanus</i>	Espinal, Aguada	Aug/20/08	Aug/22/08	0	-----	-----	----	----
Sciaenidae	<i>M. americanus</i>	Guanajibo, Mayagüez	Jan/17/09	Jul/21/09	0	-----	-----	----	----
Sciaenidae	<i>M. americanus</i>	Guanajibo, Mayagüez	Jan/17/09	Jul/21/09	1	Monogenea: Polyonchoinea	Not identified	5	
Sciaenidae	<i>Micropogonias furnieri</i>	Guanajibo, Mayaguez	Jan/17/09	Jan/21/09	2	Monogenea: Macrovalvitrematidae	<i>Macrovalvitrema argentinensis</i> <i>Pterinotrematoides avaginata</i>	1 3	11 12-14
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayaguez	Jan/17/09	Jan/23/09	1	Monogenea: Macrovalvitrematidae	<i>P. avaginata</i>	2	15, 85
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayaguez	Jan/17/09	Jan/28/09	2	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i> <i>P. avaginata</i>	2 2 (1 L)	16-17 V I-A2
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayaguez	Jan/17/09	Feb/11/09	1	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i>	1	18
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayaguez	Jan/17/09	Jul/1/09	2	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i> <i>P. avaginata</i>	1 1	45 V I-C6
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayaguez	Jan/17/09	Aug/31/09	1	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i>	1	46
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayaguez	Jan/17/09	Aug/31/09	1	Annelida: Hirudinea	<i>Trachelobdella lubrica</i>	1	V III-A5
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayaguez	Jan/17/09	Aug/31/09	2	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i> <i>P. avaginata</i>	1 1	47 V I-C8 (L)



Host family	Host species	Locality	Collection Date	Examination Date	Species richness	Parasite Taxa	Parasite Species	I or A	Slides #
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayaguez	Jan/17/09	Sep/2/09	2	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i> <i>P. avaginata</i>	1 1	48 V I-C9 (L)
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayaguez	Jan/17/09	Oct/5/09	1	Monogenea: Macrovalvitrematidae	<i>P. avaginata</i>	1	V I-C10 (L)
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayaguez	Jan/17/09	Jan/15/10	1	Monogenea: Macrovalvitrematidae	<i>P. avaginata</i>	1	49
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayaguez	Jan/17/09	Jan/26/10	1	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i>	2	50, V I-D2
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayaguez	Jan/17/09	Aug/26/10	2	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i> <i>P. avaginata</i>	1 1	Used for SEM
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayaguez	Jan/17/09	Sept/10/10	2	Monogenea: Macrovalvitrematidae	<i>P. avaginata</i>	1 2	V II-B2 95, V II-B2
Sciaenidae	<i>M. furnieri</i>	Boquilla, Mayaguez	Jan/29/09	Mar/26/09	1	Monogenea: Macrovalvitrematidae	<i>P. avaginata</i>	1	23
Sciaenidae	<i>M. furnieri</i>	Boquilla, Mayaguez	Jan/29/09	Oct/15/10	1	Monogenea: Macrovalvitrematidae	<i>P. avaginata</i>	1	Small vial
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayagüez	Feb/5/09	Mar/10/09	1	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i>	1	21
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayagüez	Feb/5/09	Mar/11/09	1	Monogenea: Macrovalvitrematidae	<i>P. avaginata</i>	1 (L)	V I-A9
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayagüez	Feb/5/09	Mar/16/09	1	Monogenea: Macrovalvitrematidae	<i>P. avaginata</i>	4	22, CB-2-3
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayagüez	Feb/5/09	Mar/17/09	1	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i>	1	87
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayaguez	Feb/5/09	Mar/17/09	3	Annelida: Hirudinea Digenea Monogenea: Macrovalvitrematidae	<i>T. lubrica</i> not identified <i>P. avaginata</i>	1 1 5	V III-3 V III-2 8, CB-4-7
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayaguez	Feb/15/09	Feb/17/09	2	Monogenea: Macrovalvitrematidae	<i>P. avaginata</i>	2	19, CB-1

Host family	Host species	Locality	Collection Date	Examination Date	Species richness	Parasite Taxa	Parasite Species	I or A	Slides #
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayaguez	Feb/15/09	Feb/17/09	1	Monogenea: Macrovalvitrematidae	<i>P. avaginata</i>	2	20, V I-A5
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayaguez	Feb/15/09	Feb/17/09	1	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i>	1	86
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayaguez	Feb/4/09	Feb/10/09	1	Annelida:Hirudinella	<i>Trachelobdella lubrica</i>	1	VIII-1
Sciaenidae	<i>M. furnieri</i>	Boquilla, Mayagüez	Mar/31/09	Apr/14/09	2	Annelida: Hirudinea Monogenea: Macrovalvitrematidae	<i>T. lubrica</i> <i>P. avaginata</i>	1 1	V III-A4 9
Sciaenidae	<i>M. furnieri</i>	Boquilla, Mayagüez	Mar/31/09	May/2/09	3	Nematoda Monogenea: Macrovalvitrematidae	not identified <i>M. argentinensis</i> <i>P. avaginata</i>	2 1 2	An box 1-2 44 10,43
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayagüez	Apr/18/09	Jun/7/10	1?	Monogenea: Macrovalvitrematidae	<i>P. avaginata</i>	9 or 10	66-69, 89-90, V II-A4
Sciaenidae	<i>M. furnieri</i>	Loíza	Jul/30/09	Jul/26/10	1	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i>	1	93
Sciaenidae	<i>M. furnieri</i>	Loíza	Jul/30/09	Jul/26/10	1	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i>	1	73
Sciaenidae	<i>M. furnieri</i>	Loíza	Jul/30/09	Jul/27/10	1	Monogenea: Macrovalvitrematidae	? <i>M. argentinensis</i>	1	94
Sciaenidae	<i>M. furnieri</i>	Loíza	Jul/30/09	Jul/28/10	1	Monogenea: Macrovalvitrematidae	? <i>M. argentinensis</i>	4	74, V II-B1
Sciaenidae	<i>M. furnieri</i>	Loíza	Jul/30/09	Sept/10/10	2	Annelida: Hirudinea Monogenea: Macrovalvitrematidae	Not identified ? <i>M. argentinensis</i>	7	V III-A10 82, 96, V II-B3, II-B4 (alcohol)
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayagüez	Jan/14/11	Jan/14/11	2	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i> <i>P. avaginata</i>	2 (1d) 4(1,2d)	V II-B8 V II-B9

Host family	Host species	Locality	Collection Date	Examination Date	Species richness	Parasite Taxa	Parasite Species	I or A	Slides #
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayagüez	Jan/14/11	Jan/14/11	2	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i> <i>P. avaginata</i>	1 10	Lost during staining 98-99, V II-B10
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayagüez	Jan/15/11	Jan/15/11	2	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i> <i>P. avaginata</i>	1 2 (1L)	Damaged during ovoposition
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayagüez	Jan/22/11	Jan/22/11	2	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i> <i>P. avaginata</i>	1 4	V II-C7 V II-C9
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayagüez	Jan/22/11	Jan/22/11	2	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i> <i>P. avaginata</i>	3 (1L) 2	Damaged during ovoposition
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayagüez	Jan/22/11	Jan/22/11	2	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i> <i>P. avaginata</i>	2 5	V II-C8 V II-C10
Sciaenidae	<i>M. furnieri</i>	Guanajibo, Mayagüez	Jan/22/11	Jan/22/11	2	Monogenea: Macrovalvitrematidae	<i>M. argentinensis</i> <i>P. avaginata</i>	2 2	*Not completed
Sciaenidae	<i>Umbrina broussonneti</i>	Espinal, Aguada	Sept/15/08	Sept/15/08	1	Monogenea: Polyonchoinean	<i>Rhamnocerchus</i> sp.	8	V III-B5
Sciaenidae	<i>Umbrina broussonneti</i>	Espinal, Aguada	Sept/15/08	Sept/15/08	1	Monogenea: Polyonchoinean	<i>Rhamnocerchus</i> sp.	5	V III-B6
Sciaenidae	<i>Umbrina broussonneti</i>	Espinal, Aguada	Aug/20/08	Aug/22/08	---	-----	-----	---	---
Sciaenidae	<i>Umbrina broussonneti</i>	Espinal, Aguada	Aug/20/08	Aug/22/08	1	Digenea	Not identified	1	
Sciaenidae	<i>Umbrina broussonneti</i>	Boquilla, Mayagüez	Mar/18/09	Jul/23/10	1	Monogenea: Polyonchoinea	Not identified	2	V III-F5
Sciaenidae	<i>U. coroides</i>	Boquilla, Mayagüez	Mar/31/09	Apr/29/09	1	Monogenea: Polyonchoinean	Not identified	13	V III-B2

Host family	Host species	Locality	Collection Date	Examination Date	Species richness	Parasite Taxa	Parasite Species	I or A	Slides #
Sciaenidae	<i>U. coroides</i>	Boquilla, Mayagüez	Mar/31/09	Apr/29/09	1	Monogenea: Polyonchoinean	Not identified	6	V III-B4
Sciaenidae	<i>U. coroides</i>	Boquilla, Mayagüez	Mar/31/09	Jul/27/10	0	-----	-----	----	----
Sciaenidae	<i>Stellifer colonensis</i>	Guanajibo, Mayagüez	Jan/17/01	Jan/ 19/09	0	-----	-----	----	----
Sciaenidae	<i>S. colonensis</i>	Guanajibo, Mayagüez	Jan/17/01	Jan/ 19/09	0	-----	-----	----	----
Sciaenidae	<i>S. colonensis</i>	Guanajibo, Mayagüez	Jan/17/09	Jul/26/10	0	-----	-----	----	----
Sciaenidae	<i>S. colonensis</i>	Boquilla, Mayagüez	May/11/09	Jul/26/10	0	-----	-----	----	----
Sciaenidae	<i>S. colonensis</i>	Boquilla, Mayagüez	May/11/09	Jul/26/10	0	-----	-----	----	----
Haemulidae	<i>Conodon nobilis</i>	Boquilla, Mayagüez	Jan/27/09	Jun/9/10	0	-----	-----	----	----
Haemulidae	<i>C. nobilis</i>	Boquilla, Mayagüez	Jan/27/09	Jun/9/10	0	-----	-----	----	----
Haemulidae	<i>C. nobilis</i>	Boquilla, Mayagüez	Jan/27/09	Jun/9/10	0	-----	-----	----	----
Haemulidae	<i>C. nobilis</i>	Boquilla, Mayagüez	Feb/4/09	Jun/21/09	1	Digenea	Not identified	1	V III-E4
Haemulidae	<i>C. nobilis</i>	Boquilla, Mayagüez	Feb/4/09	Jun/21/09	0	-----	-----	----	----
Haemulidae	<i>Haemulon aurolineatum</i>	Combate, Cabo Rojo	Jun/26/09	Feb/9/10	0	-----	-----	----	----
Haemulidae	<i>H. aurolineatum</i>	Combate, Cabo Rojo	Aug/6/09	Jun/21/10	3	Isopoda	<i>Gnathia</i> sp.	2	V III-D7
						Digenea	Not identified	1	V III-E3
						Monogenea: Polyonchoinea	Not identified	1	V III-D7
Haemulidae	<i>H. aurolineatum</i>	Combate, Cabo Rojo	Aug/6/09	Jul/28/10	0	-----	-----	----	----
Haemulidae	<i>H. parra</i>	Combate, Cabo Rojo	Aug/6/09	Jun/11/10	1	Monogenea: Polyonchoinea	Not identified	1	V III-G1

Host family	Host species	Locality	Collection Date	Examination Date	Species richness	Parasite Taxa	Parasite Species	I or A	Slides #
Haemulidae	<i>H. parra</i>	Combate, Cabo Rojo	Aug/6/09	Jul/27/10	1	Nematoda	Not identified	1	V III-G?
Haemulidae	<i>H. parra</i>	Combate, Cabo Rojo	Aug/6/09	Jul/27/10	0	-----	-----	----	-----
Haemulidae	<i>H. plumieri</i>	Combate, Cabo Rojo	Aug/6/09	Jun/17/09	3	Cestoda Monogenea: Polyonchoinea ?	Larva not identified  Not identified Egg (larger than polyonchoineans)	1  3 1	V III-G4  V III-G2 V III-G3
Haemulidae	<i>H. plumieri</i>	Combate, Cabo Rojo	Aug/6/09	Dec/18/09	1	Monogenea: Polyonchoinea	Not identified	14	V I-D6
Haemulidae	<i>H. plumieri</i>	Combate, Cabo Rojo	Aug/6/09	Jul/28/10	1	Annelida: Hirudinea	Not identified	1	
Haemulidae	<i>H. sciurus</i>	Combate, Cabo Rojo	Jul/ 23/09	Jun/8/10	1	Monogenea: Polyonchoinea	Not identified	1	Lost
Haemulidae	<i>H. sciurus</i>	Combate, Cabo Rojo	Jun/26/09	Dec/8/09	1	Copepoda	Not identified	1	V III-D1
Haemulidae	<i>H. sciurus</i>	Combate, Cabo Rojo	Jun/26/09	Dec/8/09	1	Monogenea: Polyonchoinea	Not identified	4-5	V I-D8
Haemulidae	<i>H. sciurus</i>	Combate, Cabo Rojo	Aug/6/09	Jun/8/10	0	-----	-----	----	-----
Haemulidae	<i>H. sciurus</i>	Combate, Cabo Rojo	Aug/6/09	Jun/11/10	0	-----	-----	----	-----
Haemulidae	<i>H. sciurus</i>	Combate, Cabo Rojo	Aug/6/09	Jun/21/10	2	Monogenea: Polyonchoinea	Not identified Not identified	1 1	V III-F3 V III-F3
Haemulidae	<i>H. steindachneri</i>	Loíza	Jul/30/09	Jul/28/10	1	Copepoda	Not identified	1	
Haemulidae	<i>Pomadasys corvinaeformis</i>	Boquilla, Mayagüez	Mar/16/09	Feb/9/10	1	Monogenea: Macrovalvitrematidae	<i>Pseudotagia</i> n. sp.	1	
Haemulidae	<i>P. corvinaeformis</i>	Boquilla, Mayagüez	Mar/18/09	Feb/9/10	2	Annelida: Hirudinea Monogenea: Macrovalvitrematidae	Not identified  <i>Pseudotagia</i> n. sp.	1  3	V III-A7  CB-8, V I-D4

Host family	Host species	Locality	Collection Date	Examination Date	Species richness	Parasite Taxa	Parasite Species	I or A	Slides #
Haemulidae	<i>P. corvinaeformis</i>	Boquilla, Mayagüez	Mar/31/09	Apr/27/09	2	Monogenea: Macrovalvitrematidae Diclidophoridae	<i>Pseudotagia</i> n.sp <i>Eurysorchis</i> sp.	3 2	24-26 Both sl 27
Haemulidae	<i>P. corvinaeformis</i>	Boquilla, Mayagüez	Mar/31/09	Apr/27/09	3	Monogenea: Macrovalvitrematidae Diclidophoridae Polyonchoinea	<i>Pseudotagia</i> n.sp Chorichotylinae sp. Not identified	4 1 1	28-29, V I-B7 30 V III-B1
Haemulidae	<i>P. corvinaeformis</i>	Boquilla, Mayagüez	Mar/31/09	Apr/27/09	2	Monogenea: Macrovalvitrematidae Diclidophoridae	<i>Pseudotagia</i> n.sp Chorichotylinae sp.	1 1	31 B9(L)
Haemulidae	<i>P. corvinaeformis</i>	Boquilla, Mayagüez	Mar/31/09	Apr/27/09	2	Monogenea: Macrovalvitrematidae  Diclidophoridae Unknown	<i>Pseudotagia</i> n.sp  Chorichotylinae sp. egg	6  1 1	32-34, V I-B10 35 V I-C2
Haemulidae	<i>P. corvinaeformis</i>	Boquilla, Mayagüez	Mar/31/09	Apr/28/09	1	Monogenea: Macrovalvitrematidae	<i>Pseudotagia</i> n. sp.	4	36-37, V I-C3
Haemulidae	<i>P. corvinaeformis</i>	Boquilla, Mayagüez	Mar/31/09	Apr/28/09	1	Monogenea: Macrovalvitrematidae	<i>Pseudotagia</i> n. sp.	4	38, V I-C3
Haemulidae	<i>P. corvinaeformis</i>	Boquilla, Mayagüez	Mar/31/09	Apr/28/09	1	Monogenea: Macrovalvitrematidae	<i>Pseudotagia</i> n. sp.	3	39, 84, V I C3
Haemulidae	<i>P. corvinaeformis</i>	Boquilla, Mayagüez	Mar/31/09	May/5/09	1	Monogenea: Macrovalvitrematidae	<i>Pseudotagia</i> n. sp.	10	40-42, 83, V I-C4
Haemulidae	<i>P. corvinaeformis</i>	Loiza	Julio/30/09	Jun/2/10	0	-----	-----	----	
Haemulidae	<i>P. corvinaeformis</i>	Loiza	Julio/30/09	Jun/2/10	2	Annelida: Hirudinea Monogenea: Macrovalvitrematidae	Not identified <i>Pseudotagia</i> n. sp	1 2	V III-A9 56, V I-D9
Haemulidae	<i>P. corvinaeformis</i>	Loiza	Julio/30/09	Jun/3/09	1	Monogenea: Macrovalvitrematidae	<i>Pseudotagia</i> n. sp.	2	57, V I-D10

Host family	Host species	Locality	Collection Date	Examination Date	Species richness	Parasite Taxa	Parasite Species	I or A	Slides #
Haemulidae	<i>P. corvinaeformis</i>	Loiza	Julio/30/09	Jun/3/09	4	Monogenea: Macrovalvitrematidae  Diclidophoridae  Unknown	<i>Pseudotagia</i> n.sp  <i>Eurysorchis</i> sp. Choricothylineae sp. Unknown	6  1 3 1	58-61, V II-A1 62 63-64, V II-A2
Haemulidae	<i>P. corvinaeformis</i>	Guanajibo, Mayagüez	Jan/22/11	Jan/11/22	0	-----	-----	-----	-----
Haemulidae	<i>P. corvinaeformis</i>	Guanajibo, Mayagüez	Jan/22/11	Jan/11/22	0	-----	-----	-----	-----
Haemulidae	<i>P. corvinaeformis</i>	Guanajibo, Mayagüez	Jan/22/11	Jan/11/22	0	-----	-----	-----	-----
Haemulidae	<i>P. corvinaeformis</i>	Guanajibo, Mayagüez	Jan/22/11	Jan/11/22	1	Monogenea: Diclidophoridae	Choricothylineae sp.	1	V I-C5
Sparidae	<i>Archosargus rhomboidalis</i>	Parguera, Lajas	Sept/2/08	Sept/3/08	0	-----	-----	-----	-----
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Jun/26/09	Jun/6/09	1	Monogenea: Polyonchoinea	Not identified	1	
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Jun/26/09	Jan/2/10	1	Annelida: Hirudinea	Not identified	1	V III-A6
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Jun/26/09	Feb/2/10	3	Annelida: Hirudinea Monogenea: Macrovalvitrematidae Polyonchoinea	Not identified Genus N., n. sp. Not identified	1 1 1	¿? 51 V II-D7
Sparidae	<i>A. rhomboidalis</i>			Apr/13/10	1	Monogenea: Macrovalvitrematidae	Genus N., n. sp.	2	52
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Jul/10/09	May/26/10	1	Monogenea: Polyonchoinea	Not identified	2	V III-B
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Jul/10/09	May/26/10	3	Cestoda Copepoda Monogenea: Polyonchoinea	Not identified Not identified Not identified	5-6 2 7	V III-C V III-D V III-B
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Jul/10/09	May/26/10	2	Copepoda Monogenea: Polyonchoinea	Not identified Not identified	4 1	

Host family	Host species	Locality	Collection Date	Examination Date	Species richness	Parasite Taxa	Parasite Species	I or A	Slides #
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Jul/ 23/09	Jun/1/09	2	Copepoda	Not identified	1	1
						Monogenea: Macrovalvitrematidae	Gen N., n. sp.	2	2V III-D4
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Jul/ 23/09	Jun/2/10	0	-----	-----	-----	-----
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Jul/ 23/09	Jun/2/10	4	Annelida: Hirudinea	Not identified	1	V III-A8
						Copepoda	Not identified	1	V III-D5
						Monogenea:			
						Macrovalvitrematidae	Gen. N.,n. sp.	1	55
						Microcotylidae	<i>Microcotyle archosargus</i>	1	V III-F1
						Unknown	1	1	V I-D8
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Jul/ 23/09	Jun/7/10	2	Cestoda	Larvae not identified	4	V III-E2
						Monogenea: Macrovalvitrematidae	Gen. N., n.sp.	2	70, V II-A5
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Jul/ 23/09	Jun/7/10	0	-----	-----	----	-----
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Jul/ 23/09	Jun/7/10	2	Isopoda	<i>Gnathia</i> sp.	1	
						Monogenea: Polyonchoinea	Not identified	1	
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Jul/ 23/09	Jun/8/10	2	Isopoda	<i>Gnathia</i> sp.	1	V III-D6
						Monogenea: Macrovalvitrematidae	Gen. N., n. sp.	4	71-72, 91-92
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Jul/31/09	Jul/27/10	1	Monogenea: Macrovalvitrematidae	Gen. N., n. sp.	3	V I-A10
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Jul/31/09	Sept/10/10	3	Monogenea: Macrovalvitrematidae	Gen. N. n. sp.	2	V II-B5 (AI), VII-B6
							Gen N. n. sp Not opisthaptor	1	VII-B6
						Polyonchoinea	Not identified	2	VII-B6
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo		Oct/6/10* Damaris	1	Monogenea: Macrovalvitrematidae	Gen. N., n. sp.	1	Lost during staining



Host family	Host species	Locality	Collection Date	Examination Date	Species richness	Parasite Taxa	Parasite Species	I or A	Slides #
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Jul/31/09	Nov/5-9/10 Jacob	1	Monogenea: Macrovalvitrematidae	Gen. N., n.sp.	3	Vial (alcohol)
Sparidae	<i>A. rhomboidalis</i>		Jul/31/09	Nov/18/10 Jacob	1	Monogenea: Macrovalvitrematidae	Gen. N., n.sp.	1	Vial (formalin)
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo		Nov/19/10* Damaris	2	Monogenea: Macrovalvitrematidae Polyonchoinea	Gen. N., n. sp. Not identified	1 4	100
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Dec/4/10	Dec/4/10	1	Monogenea: Macrovalvitrematidae	Gen. N., n.sp.	1	V I-B7
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Dec/4/10	Dec/4/10	0	-----	-----	----	-----
Sparidae	<i>A. rhomboidalis</i>	Combate, Cabo Rojo	Dec/4/10	Dec/4/10	0	-----	-----	----	-----
Sparidae	<i>Calamus penna</i>	Combate, Cabo Rojo	Jun/26/09	Jul/21/10	2	Isopoda Digenea	<i>Gnathia</i> sp. Not identified	1 1	
Sparidae	<i>C. penna</i>	Combate, Cabo Rojo	Jun/26/09	Jul/21/10	0	-----	-----	----	-----
Sparidae	<i>C. penna</i>	Combate, Cabo Rojo	Jun/26/09	Jul/26/10	1	Monogenea: Polyonchoinea	Not identified	1	Lost
Sparidae	<i>C. pennatula</i>	Combate, Cabo Rojo	Jun/26/09	Sept/1/09	1	Monogenea: Polyonchoinea	Not identified	3	V III-B7
Sparidae	<i>C. pennatula</i>	Combate, Cabo Rojo	Jun/26/09	Sept/14/09	1	Monogenea: Polyonchoinea	Not identified	1	V I-D9
Sparidae	<i>C. pennatula</i>	Combate, Cabo Rojo	Jun/26/09	Sept/14/09	1	Monogenea: Polyonchoinea	Not identified	1	V I-D10
Sparidae	<i>C. pennatula</i>	Combate, Cabo Rojo	Jun/26/09	Jul/21/10	0	-----	-----	----	-----