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Contributions to the
Eleventh International Symposium on Flatworm Biology
Diepenbeek (Belgium) 26-31 July 2009

INTRODUCTION

The XIth International Symposium on Flatworm Biology closed half a century of symposia that focussed on turbellarian research. The first two of these were held in memory of two icons of turbellarian research: the "Libbie Hyman Memorial Symposium" (Chicago, 1970) and, seven years later, the "Alex Luther Memorial Symposium" (Tvaerminne, 1977). Three years later, the first open International Symposium on the Biology of Turbellaria was held (Diepenbeek, 1980) in honour of Tor Karling, a then still living legend in turbellarian biology. From that symposium on, an international Symposium on the Biology of Turbellaria was held every three or four years: Fredericton (Canada, 1984), Göttingen (Germany, 1987), Hirosaki (Japan, 1990); Turku (Finland, 1993), Brisbane (Australia, 1996) and Barcelona (Spain, 2000).

A short look at the abstract books and proceedings of these symposia already reveals the enormous evolution turbellarian research has gone through in all these years. Whereas most contributions in the first symposia focussed on classical morphology, taxonomy/systematics and ecology, the contributions in later symposia became more divers. With the trend set after the Brisbane symposium the symposia also covered more and more the parasitic flatworms. Contributions on stem cell biology and regeneration took a prominent place in most recent symposia, and the emergence of molecular techniques instigated new approaches in many fields of research. One of these insights led to an important change in the name of the symposia. The fact that phylogenetic analyses had clearly showed turbellarians to be paraphyletic, the Xth Symposium was called the Xth International Symposium on Flatworm Biology (ISFB). It was organised in Innsbruck by Reinhard Rieger and his group in 2006, after an interruption of seven years, reflecting Reinhard's broad interest and thorough knowledge in many fields of flatworm research, the symposium offered a wide variety of contributions. Unfortunately, Reinhard Rieger passed away a few months after the Innsbruck conference.

The XIth International Symposium of Flatworm Biology was organised in Diepenbeek (Belgium) from July 26-30, in memory of Reinhard. Coming from 18 different countries, 116 researchers attended the conference. The 42 oral contributions and 52 posters covered a broad field of subjects, from morphology, taxonomy and ecology over phylogeny to molecular signalling mechanisms, development and regeneration. Also several contributions were presented on parasitic (neodermatan) flatworm biology, which gave the opportunity to cross the "psychological border" between turbellarian and neodermatan researchers, leading to a fruitful exchange of ideas. The last day of the symposium was devoted to research on *Macrostomum* as the 3rd International *Macrostomum* meeting. This volume is not representative of the large number of contributions presented at the symposium but it clearly does represent the high diversity of topics.

The necessity of symposia as the XIth ISFB is, in our view, twofold: to give the state of the art in this field of research and to provide the opportunity to discuss and exchange ideas. Especially for young researchers it is indispensable to discuss their results with experienced specialists. As in all former symposia, many young students attended the XIth ISFB. We are sure that the same will be true at the next ISFB, that will be held in Stockhom in 2012. Hopefully, the series can continue in the 21st century, now that the younger generation has taken over.

Finally we wish to thank our sponsors and especially the Research Fondation Flanders for its financial support, the Province of Limburg and the City of Hasselt for their contribution in the social events during the symposium. The University of Hasselt is acknowledged for its support. Special thanks are due to the “crew” who made the symposium going smoothly and, last but not least, we thank Dr. Nikki Watson for correcting the manuscripts of the non-English speaking authors.

Tom Artois

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Land flatworm community structure in a subtropical deciduous forest in Southern Brazil

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ABSTRACT. Due to their biological characteristics and habitat requirements, land planarians have been proposed as indicator taxa in biodiversity and conservation studies. Herein, we investigated spatial patterns of land flatworm communities in the three main existent vegetation types of the most significant remnant of the subtropical deciduous forest in south Brazil. The main questions were: (1) How species-rich is the study area? (2) How are community-structure attributes allocated in areas with distinct floristic composition? (3) What are the effects of soil humidity and soil organic matter content on flatworm abundance? (4) Are there seasonal differences regarding species richness? (5) Are the communities in the three types of vegetation distinct? Twenty-two flatworm species, distributed in five genera and two subfamilies, were recorded. Results indicated that: (1) species richness, evenness, abundance, and dominance in the three vegetation types are not significantly different; (2) diversity indices were higher for areas with caducifolious forest than for areas with jaboticaba trees with marginally significant differences; (3) flatworm abundance is negatively related to soil organic matter; (4) there are no significant differences in flatworm species richness throughout the year; and (5) flatworm communities in the three types of vegetation are not distinct. Soil organic matter content was abundant at sites with distinct vegetation types, thereby negatively affecting flatworm abundance. The original vegetation of the study area has been well-preserved, which may be an explanation for the absence of a clear separation of the flatworm communities of the three main types of vegetation in the study area.

KEY WORDS: diversity, conservation unit, spatial patterns, soil organic matter contents, triclads

INTRODUCTION

Due to their biological characteristics and habitat requirements, land planarians have been proposed as indicator taxa in biodiversity and conservation studies (Sluys, 1998). They are top predators within the soil ecosystem, preying on other invertebrates (Du Bois-Reymond Marcus, 1951; Ogren, 1995; Jones & Cumming, 1998; Sluys, 1999; Leal-Zanchet & Carbayo, 2001; Carbayo & Leal-Zanchet, 2003; Prasniski & Leal-Zanchet, 2009). As land flatworms are not adapted to conserve water (Kawaguti, 1932), they are sensitive to the humidity and temperature conditions of their environment (Froehlich, 1955; Winsor et al., 1998; Sluys, 1998, 1999).

From studies on land flatworm community structure, there are indications that they are sensitive to replacement of the natural habitat by plantations with exotic species. Their diversity is inversely related to the degree of habitat disturbance (Carbayo et al., 2001, 2002; Leal-Zanchet et al., 2006; Fonseca et al., 2009). After having studied land flatworm community structure in two types of ombrophilous forest, Fick et al. (2006) showed that the communities were clearly distinct, and suggested that they are not affected by the edge-effects of their habitats.

Investigations on land planarian diversity in forest ecosystems have been concentrated in areas of ombrophilous forest located in the northeast of southern Brazil (Leal-Zanchet & Carbayo, 2000, 2001; Carbayo et al., 2001, 2002; Fick et al. 2003, 2006; Palacios et al., 2006; Baptista et al., 2006; Leal-Zanchet et al., 2006; Leal-Zanchet & Baptista, 2009). These studies have registered high species richness, much of it represented by new taxa (Leal-Zanchet & Carbayo, 2001; Carbayo & Leal-Zanchet, 2001, 2003; Froehlich & Leal-Zanchet, 2003; Baptista & Leal-Zanchet, 2005; Leal-Zanchet & Froehlich, 2006; Lemos & Leal-Zanchet, 2008). In deciduous and semi-deciduous forests, land flatworm communities have been studied only in small remnants located in the central and northeastern regions of the state of Rio Grande do Sul (Southern Brazil), respectively. In those areas, relatively low species richness has been registered (Castro & Leal-Zanchet, 2005; Antunes et al., 2008).

In the past, the largest forest-type in southern Brazil consisted of deciduous forest. What remains now occupies only 23.8% of the originally occupied area and is mainly located in the northwestern and central regions. According to Irgang (1980), the northwestern part, corresponding to 56% of the total area, represents the sole significant remnant. It is represented by the Turvo State Park, the largest wholly-protected regional conservation unit, being entirely disconnected from the other forest remnants (IBGE, 1986; SEMA/DEFAP, 2005). This Park is also the main wildlife refuge in the region, sheltering a rich fauna. It includes all the large felids that are characteristic to meridional Brazil

(Lema, 1980; Kasper et al., 2007). Nevertheless, there is a lack of data on fauna community structure, giving rise to concern as to its conservation (Albuquerque, 1977, 1985; Kasper et al., 2007).

Tropical and subtropical deciduous forest biomes developed in response not to seasonal temperature variations, but to seasonal rainfall patterns. In moist deciduous forests, overall rainfall is heavy, with a warm and wet summer and cooler temperatures in the winter. Some trees lose their leaves during the cooler winter. Typically, trees do not stand as close together as in tropical rainforests, thus facilitating the penetration of sunlight, especially when deciduous species are without leaves. This penetration of sunlight down to the forest floor gives rise to dense undergrowth.

In the present study, we investigated the land flatworm community in the most significant remnant of deciduous forest in southern Brazil, the Turvo State Park. Our main research questions were: (1) How species-rich is the study area; (2) How are community-structure attributes allocated in areas with distinct floristic composition; (3) Is flatworm abundance affected by soil humidity and soil organic matter content; (4) Are there seasonal differences regarding species richness; (5) Are the communities in the three types of vegetation distinct?

MATERIALS AND METHODS

Study area

The Turvo State Park (Fig. 1) is located in the northwest of the state of Rio Grande do Sul, southern Brazil (27º00'-27º20'S; 53º40'-54º10W), and covers an area of 17,491.40ha. It is bounded in the north by the Uruguay River, in the south by private landholdings as well as the Calixto and Bonifácio Rivers, in the east by the Parizinho River, and in the west by the Turvo River. Coverage consists mainly of deciduous forest (IBGE, 1986), with well-developed areas of primary vegetation made up of more than 700 species belonging to 121 families, predominantly the Bignoniaceae, Asteraceae, Euphorbiaceae, Myrtaceae, Fabaceae, and Rubiaceae. The structure of the south Brazilian deciduous forest is represented mainly by two distinct tree strata. The emergent stratum is formed by deciduous trees with heights ranging from 16 to 30 meters. The second stratum is constituted mainly of evergreen species with heights ranging from 10 to 16 meters. In addition, there is a third stratum of young, low trees (IBGE, 1986; Vasconcellos et al., 1992; A. Backes, personal communication). The physiognomy of this type of deciduous forest is defined by a canopy dominated by caducifolious species (IBGE, 1986). The Turvo State Park also contains areas of secondary vegetation, which were formerly used for farming, occurring in the northwestern and northeastern parts. These are characterized by thinner and smaller trees and a dense understory. There are also large areas in the Park with jaboticaba trees (*Myrciaria trunciflora* Berg), characterized by an understory with few seedlings. They occur in areas with insufficiently drained, very humid soils (SEMA/DEFAP, 2005).

The climate is warm-temperate (subtropical) and humid, without any marked dry periods (Nimer, 1989). The average annual precipitation is about 1,900mm year^{-1}, with the heaviest rains occurring in the summer (SEMA/DEFAP, 2005). Soils are mainly clayey, dark-red in color, shallow and quickly drained.

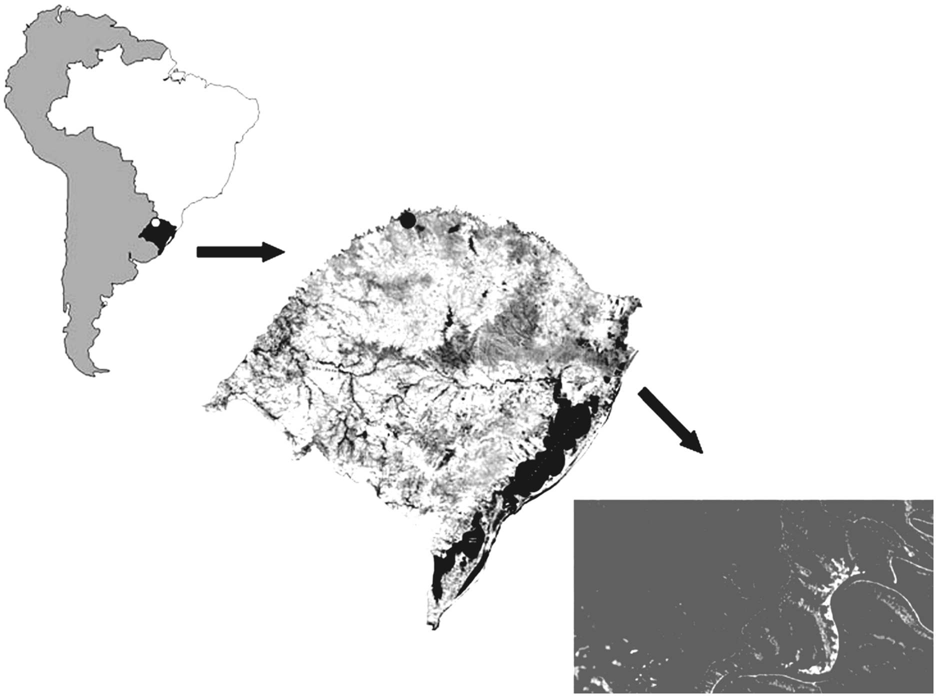

Fig. 1. – Location map of the Turvo State Park in southern Brazil and sampling sites. JI-III: areas with jaboticaba trees; PI-PIII: areas with primary vegetation (deciduous forest); SI-SIII: areas with secondary vegetation (deciduous forest).

Methods

Surveys were carried out in three sites of the following types of vegetation: (1) areas of caducifolious forest with primary vegetation (PI, PII, and PIII) located in the northeast, northwest, and close to the center of the Park, respectively; (2) areas of caducifolious forest with secondary vegetation (SI, SII, and SIII), all located in the southwest; and (3) areas with jaboticaba trees (JI, JII, and JIII), located approximately in the southeast (JI and JII) and central regions (JIII) (Fig. 1). Samples were taken by five experienced collectors, twice per season, from May 2005 through November 2006, amounting to a total of eight samplings. Each sampling lasted three days. In each site ten plots (50m x 2m) were set up. Five of them were randomly selected for each sampling. We searched, at daylight, for flatworms by means of direct sampling in soil litter, under and inside fallen logs and branches, and under rocks. The sampling effort was 1 hour per plot. After inspection, the branches, logs, and rocks were returned to their original positions , in order to avoid alteration of soil microhabitats (Ball & Reynoldson, 1981; Winsor, 1997).

We registered the relative humidity of the air at each site with a hygrometer, avoiding rainy days, from October 2005 to November 2006. Soil samples (60g) were collected from each site by means of a pycnometer in order to analyse humidity and organic matter content. This was done once in March 2006.

For identification we used techniques described by Leal-Zanchet & Carbayo (2001) and Seitenfus & Leal-Zanchet (2004). Adult specimens without all the necessary morphological characteristics required for identification at the genus level were placed in the collective group *Pseudogeoplana* Ogren & Kawakatsu. 1990. Specimens registered as ”unidentified” were immature specimens or animals lost before fixation.

Abundance, species richness, dominance, evenness, and the Shannon diversity index (*H'*, see Krebs, 1989) for each area were calculated and the data obtained were log-transformed (log-linear). The ANOVA, followed by Tukey, was applied for comparing habitats. Repeated measures ANOVA was used to verify seasonal distribution of richness. Both forms of variance analysis were calculated using Systat 11.0 (Systat Software Inc., Richmond, OR, USA). Unidentified specimens were considered only for abundance analysis. Collectors curve based on the species accumulated per sampling and the probable number of species (Jackknife Estimators of Species Richness) were calculated using PC-ORD (McCune & Mefford, 1999).

TABLE 1

Abundance of land flatworms in areas with jaboticaba trees (JI-III), areas with deciduous forest (primary vegetation) (PI-III), and areas with regenerating deciduous forest (secondary vegetation) (SI-III) in the Turvo State Park, southern Brazil.

Species	PI	PII	PIII	SI	SII	SIII	JI	JII	JIII	Total
Pasipha sp. 1	2	2	-	4	5	-	-	1	1	15
Notogynaphallia sp.	-	1	1	-	1	1	-	-	2	6
Pasipha sp. 2	1	1	1	1	-	-	-	-	-	4
Pseudogeoplana sp.27	-	4	-	-	-	-	-	-	-	4
Geoplana rubidolineata	-	-	3	-	-	-	-	-	-	3
Choeradoplana sp.	-	-	3	-	-	-	-	-	-	3
Pasipha sp.3	-	1	-	-	-	-	-	-	2	3
Geoplana sp.1	-	-	-	-	-	-	2	1	-	3
Pseudogeoplana sp.28	-	-	-	1	2	-	-	-	-	3
Pasipha sp.4	-	1	-	-	-	-	1	-	-	2
Pseudogeoplana sp.29	-	-	-	1	1	-	-	-	-	2
Rhynchodemus sp.	-	1	-	1	-	-	-	-	-	2
Geoplana sp. 2	-	1	-	-	-	-	-	-	-	1
Geoplana sp. 3	-	1	-	-	-	-	-	-	-	1
Geoplana sp. 4	1	-	-	-	-	-	-	-	-	1
Geoplana sp. 5	-	-	-	-	-	-	-	1	-	1
Geoplana sp. 6	1	-	-	-	-	-	-	-	-	1
Geoplana sp. 7	1	-	-	-	-	-	-	-	-	1
Pseudogeoplana sp.30	-	-	-	1	-	-	-	-	-	1
Pseudogeoplana sp.31	-	-	-	1	-	-	-	-	-	1
Pseudogeoplana sp.32	1	-	-	-	-	-	-	-	-	1
Pseudogeoplana sp.33	-	1	-	-	-	-	-	-	-	1
Unidentified specimens	1	1	2	1	-	-	1	1	-	7
Richness	6	10	4	7	4	1	2	3	3	22
Abundance	8	15	10	11	9	1	4	4	5	67

To describe the community spatial pattern, we used Cluster analysis (Ward's linkage) for the presence and absence of species. To test the relationship between abundance and both air and soil humidity, and organic matter contents, we used stepwise multiple regression analysis. Both analyses were performed employing Systat 11.0.

RESULTS

Sixty-seven specimens were found, 60 of which were identifiable, belonging to 22 species and six genera of the two subfamilies Geoplaninae and Rhynchodeminae (Table 1).

Overall abundance was higher in areas with primary vegetation, less in secondary vegetation and still less in those with jaboticaba trees (Tables 1 and 2), although differences between the three were not significant (ANOVA, $F_{(2,6)=}$1.201; p>0.05). The most abundant species were *Pasipha* sp. 1 and *Notogynaphallia* sp., registered in the three vegetation types. Many species (approx. 60% of the total) were represented by less than three individuals, and 10 were even singletons. Sixteen species, or approx. 73% of all those recorded, were unique, occurring exclusively in only one of the three vegetation types. Most of these were scarce and represented by only one to three individuals. One unique (*Pseudogeoplana* sp.27), represented by 4 individuals, was moderately abundant (Table 1).

TABLE 2
Land flatworm community-structure attributes in areas with jaboticaba trees, areas with deciduous forest (primary vegetation), and areas with regenerating deciduous forest (secondary vegetation) in the Turvo State Park, southern Brazil.

Attributes	Primary vegetation	Secondary vegetation	Areas with jaboticaba-trees
Abundance	33	21	13
Species richness	18	8	6
Diversity	1.127	0.740	0.676
Evenness	0.936	0.819	0.960
Dominance	13.793	45.000	27.273

Observed species richness followed the same quantitative sequence as overall abundance, viz. first primary vegetation, then secondary vegetation and finally areas with jaboticaba trees (Tables 1 and 2), although differences between the three types were not significant (ANOVA, $F_{(2,6)}$=1.513; p>0.05). The collectors curve did not reach the asymptote (Fig. 2), as estimated species richness for the park was 33. Furthermore, on analyzing seasonal distribution of species richness, no significant differences were found between seasons (ANOVA, $F_{(2,6)}$=2.756; p>0.05).

Shannon-Wiener diversity was higher in areas with caducifolious forest than in those with jaboticaba trees (Fig. 3, Table 2), differences only being marginally significant (ANOVA, $F_{(2,6)}$=3.938; p=0.08). Regarding evenness and dominance, there were no significant differences be-

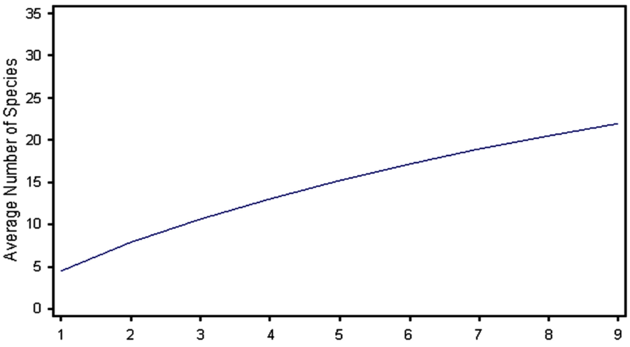

Fig. 2. – Collectors curve after eight samplings in Turvo State Park, southern Brazil.

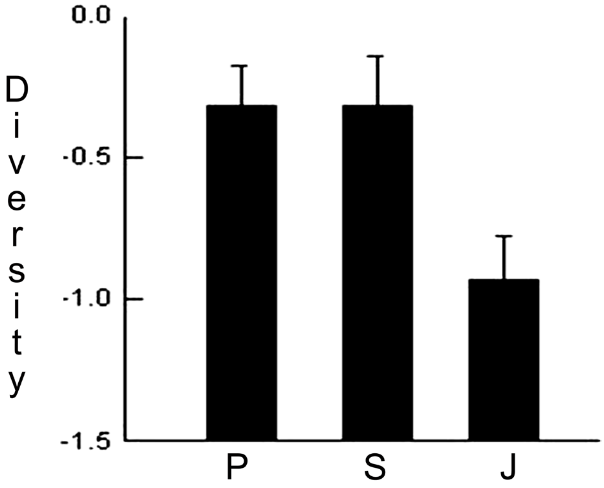

Fig. 3. – Land flatworm diversity in Turvo State Park, southern Brazil. J: areas with jaboticaba trees; P: areas with deciduous forest (primary vegetation); S: areas with regenerating deciduous forest (secondary vegetation).

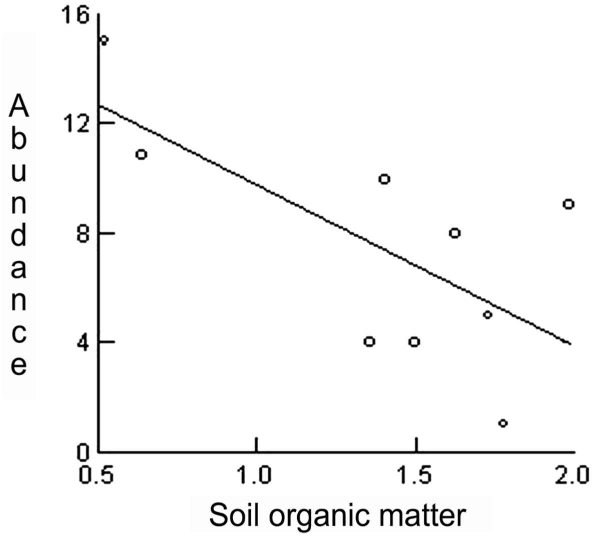

Fig. 4. – Relationship between land planarian abundance and soil organic matter content in Turvo State Park, southern Brazil.

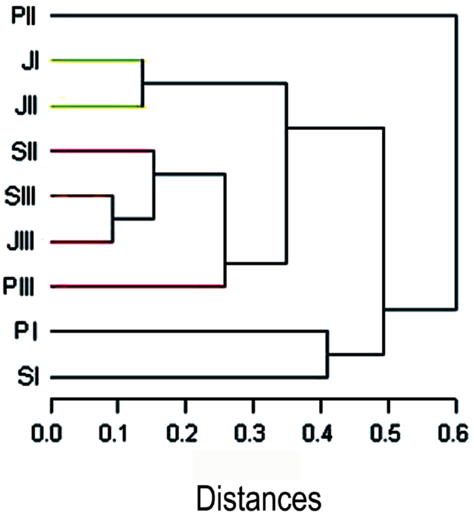

Fig. 5. – Similarity between areas with jaboticaba trees (JI-III), areas with deciduous forest (primary vegetation) (PI-III), and areas with regenerating deciduous forest (secondary vegetation) (SI-III) in Turvo State Park, southern Brazil, based on the presence and absence of land flatworm species analyzed by Cluster.

tween the three vegetation types (ANOVA, $F_{(2,6)=}$0595 and 2.665, respectively, $p>0.05$).

The abundance of land flatworms was negatively related to soil organic matter content ($F_{(1,7)}=5.856$; $r=-0.67$; $p<0.05$, Fig. 4). There was no statistically significant relationship between abundance and soil humidity ($F_{(1,7)}=0.498$; $p>0.05$) or between abundance and air humidity ($F_{(1,7)}=0.104$; $p>0.05$).

Regarding flatworm community composition, Cluster analysis demonstrated that there was high similarity between sites of distinct vegetation types (Fig. 5). Thus, flatworm communities of the different types of vegetation were not clearly distinct.

DISCUSSION

The results indicate that Turvo State Park offers refuge to a rich land planarian fauna, presenting a species richness similar to that observed in areas originally covered by araucaria forest in the northeastern part of southern Brazil (Carbayo et al., 2002). Furthermore, species richness is higher than that observed in Araucaria and Atlantic Rain Forest areas in the National Park of Aparados da Serra (Fick et al., 2003, 2006), or in areas of semi-deciduous forest (Antunes et al., 2008) located in the northeastern part of the region. Nevertheless, overall flatworm abundance in Turvo State Park is lower than that observed in the Araucaria and Atlantic Rain Forests, despite the high sampling effort in our study. In another study undertaken in relatively small remnants of deciduous forest located in the central portion of the region (Castro & Leal-Zanchet, 2005), overall abundance, as well as observed species richness were still lower, in comparison to those obtained in the present study.

Comparative analysis of the three vegetation types shows that abundance and species richness are lower in one of the sites with primary vegetation (PIII), which may be related to its proximity to the Uruguay River. The results obtained for PIII may have been negatively affected by the illegal entrance of hunters, as well as the adverse impact of tourism, which are both more intense in this part of the Park (SEMA/DEFAP, 2005). In general, flatworm diversity was not lower in areas of secondary vegetation located at the southernmost borders of the Park, and surrounded by farms, when compared to lesser disturbed areas of primary vegetation. In contrast, in areas with jaboticaba trees, which are immersed in a matrix of deciduous forest, there was the lowest value for flatworm diversity. The large number of jaboticaba trees, with their profuse fruit attracting large mammals and consequential soil compaction, could possibly negatively affect land flatworm occurrence.

Knowledge on ecological factors affecting land flatworm occurrence or abundance is extremely poor and is restricted to certain invading species of economic significance. Vegetation cover, temperature, air and soil humidity, soil texture, soil pH, soil compaction, and the presence of surface refuges may potentially constitute important factors affecting flatworm occurrence and/or abundance (Springett, 1976; Alford et al., 1998; Boag et al., 1998a, b; Sluys, 1998; Winsor, 1998; Carbayo et al., 2002; Fick et al., 2006). Intraspecific competition for food is probably the most relevant factor restricting land flatworm population size (Boag et al. 1998a), by constituting an important factor affecting both their spread and establishment. The present results indicate an additional factor, i.e. soil organic matter content, affecting flatworm abundance. It mainly derives from organic residues of dead animals, litter, dead roots and sloughed off plant cells, as well as exudates from living roots (Brown & Doube, 2004). In our study, soil organic matter content was abundant at sites with distinct vegetation types, thereby negatively affecting flatworm abundance.

Despite the existence of a certain number of scattered exotic species in the park area (SEMA/DEFAP, 2005), the original vegetation has been well-preserved. This might be an explanation for the absence of a clear separation of the flatworm communities of the three main types of vegetation in the study area. Thus, all three types of vegetation are probably offering good general conditions for various flatworm species as a consequence of the degree of preservation of the original vegetation. Similar results were found by Leal-Zanchet et al. (2006) when areas planted with *Araucaria angustifolia*, a native species, were compared to those with ombrophilous forest. However, when areas with exotic plant species were compared to those with ombrophilous forest, land flatworm communities were clearly distinct (Carbayo et al., 2002, Leal-Zanchet et al., 2006). The same was found about Araucaria and Atlantic forest (Fick et al., 2006).

The Turvo State Park harbours unique habitats as well as many invertebrate and vertebrate species (Guadagnin & Zanini, 2000; SEMA/DEFAP, 2005). It represents the last large protected area of deciduous forest immersed in a matrix of cultivated land in southern Brazil. Our results reinforce the need for preserving this natural park as an integral conservation unit.

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A new genus for seven Brazilian land planarian species, split off from *Notogynaphallia* (Platyhelminthes, Tricladida)

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ABSTRACT. Special attention is given to cephalic structures of a species complex within the *Notogynaphallia* (Platyhelminthes, Tricladida). *Notogynaphallia muelleri* is redescribed. The species possesses a cephalic musculo-glandular organ. Its glandular and muscular organization are similar to that previously observed in *N. caissara* and *N. fita* and to that herein described for *N. abundans*, *N. ceciliae*, *N. ernesti*, and *N. graffi*. Based on these unique cephalic specializations, a new genus is proposed for these seven species. Functionally, the musculo-glandular organ may be an adaptation for capturing and holding prey.

KEY WORDS: *Luteostriata* gen. nov., *Luteostriata muelleri*, cephalic retractor muscle, mesenchymal musculature, Geoplaninae, Terricola.

INTRODUCTION

In recent years, the taxonomic status of some species of *Notogynaphallia* Ogren & Kawakatsu, 1990 (Geoplaninae, Tricladida) has been discussed. The genus does not present unique characteristics, but a combination of features that are also shared with other genera of the subfamily. Indeed, Leal-Zanchet & Froehlich (2001, 2006) and Froehlich & Leal-Zanchet (2003) suggested a heterogeneous status for the genus based on certain characteristics of the reproductive organs. They also subdivided the genus into two non-formal groups according to similarities in external and internal features. One of these groups, the so-called group 2, includes *N. abundans* (Graff, 1899), *N. caissara* (Froehlich, 1955), *N. ceciliae* Froehlich & Leal-Zanchet, 2003, *N. ernesti* Leal-Zanchet & Froehlich, 2006, *N. fita* (Froehlich, 1959), *N. graffi* Leal-Zanchet & Froehlich, 2006, and *N. muelleri* (Diesing, 1861), in addition to *N. guaiana* Leal-Zanchet & Carbayo, 2001, which stands apart from the others because of the dorsal color pattern and the epithelium lining the female atrium (Leal-Zanchet & Froehlich, 2006). More recently, *N. arturi* Lemos & Leal-Zanchet, 2008 and *N. pseudoceciliae* Lemos & Leal-Zanchet, 2008 were added to this species complex.

The necessity of giving attention to the cephalic region of geoplaninid land planarians has been stressed (Carbayo & Leal-Zanchet, 2003). The finding of a cephalic musculo-glandular organ in *N. caissara* and *N. fita* once again pointed to the heterogeneity of the genus *Notogynaphallia* (Carbayo, 2006).

In the present study, we examine the cephalic region of *N. muelleri*, *N. abundans*, *N. ceciliae*, *N. ernesti*, and *N. graffi*. *Notogynaphallia guaiana* was not studied because this species does not possess cephalic musculo-glandular organs and presents a highly-developed longitudinal mesenchymal muscle system (pers. obs.). *Notogynaphallia arturi* Lemos & Leal-Zanchet, 2008 and *N. pseudoceciliae* Lemos & Leal-Zanchet, 2008 do fit into group 2, but are not included because the material was not available for the present study.

MATERIALS AND METHODS

Specimens studied are deposited in the MZU (Museu de Zoologia da Unisinos, São Leopoldo, Rio Grande do Sul, Brazil), IPP (Instituto de Pesquisas de Planárias, Unisinos), FCB (F. Carbayo´s collection, Universidade de São Paulo), and EMF (E. M. Froehlich's collection, Universidade de São Paulo). Specimens of EMF's collection histologically-processed elsewhere were assigned additional identification numbers. When possible, the external morphology was observed before and after fixation. Tissue blocks of specimens were processed following Carbayo (2006), and sectioned at 6-8 µm. The relationship between the height of sub-epidermal musculature and the height of the body (CMI = mc:h) was calculated after Froehlich (1955a). Drawings were prepared using a camera lucida.

RESULTS

TAXONOMIC SECTION

Family Geoplanidae Stimpson, 1857

Subfamily Geoplaninae Stimpson, 1857

Luteostriata gen. nov.

Diagnosis. Body slender, with parallel margins, anteriorly slightly rounded. Sensory pits in the shape of a simple invagination, encircling the cephalic end. Eyes encircling anterior end of the body. Cephalic musculo-glandular organ present; the cephalic glands of the organ open onto a U-shaped surface at the ventral region of the cephalic region; retractor muscles of the organ mainly formed by the ventral longitudinal sub-epidermal musculature. Bundle of retractor muscles lens-shaped in cross-section; muscle fibers sunken into the mesenchyma near the anterior end of the body; part of these muscles laterally traverses to the opposite side before attaching to the basement membrane. Transverse mesenchymal sub-neural muscle layer present throughout the body. Testes under the supra-intestinal mesenchymal muscle layer. Penial papilla absent. Common glandular ovovitelline duct run backwards, dorsal to the female atrium.

Type species. *Geoplana elegans* Diesing, 1861, here designated, following recommendation 69A of the CINZ (2000), since the species is herein redescribed and illustrated in detail, in addition to being common in urban areas in Blumenau, Brazil, the place of origin of the first specimens studied by Fritz Müller.

Etymology. The generic epithet refers to the yellowish, often yellow-orange (*luteus*) color of the dorsum with dark longitudinal stripes (*striatus*). The gender is female.

Luteostriata muelleri (Diesing, 1861) comb. nov.

Figs 1-9

Geoplana pallida n. sp. Schultze & Müller, 1857: 24
Geoplana mulleri nom. nov. Diesing, 1861: 511
Planaria elegans: Diesing, 1861: 511
See complete synonymies in Ogren & Kawakatsu, 1990

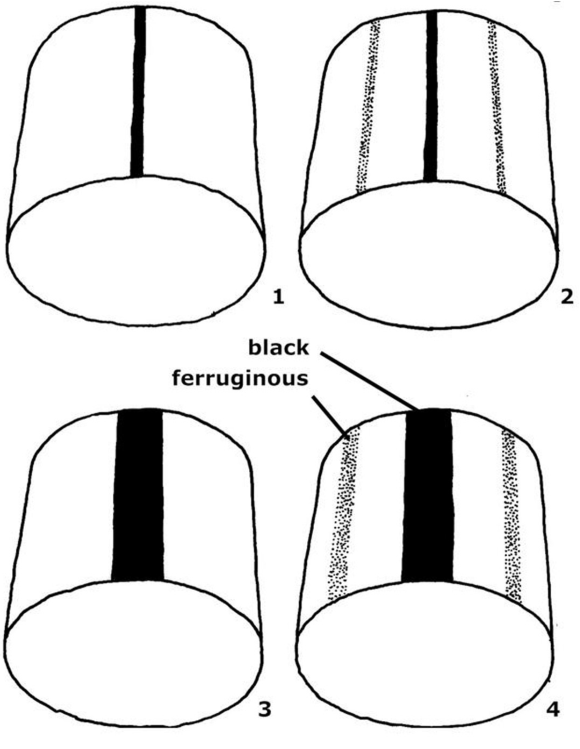
Diagnosis. Dorsal surface with a median, longitudinal black stripe; often an additional lateral ferruginous stripe on each side. Prostatic vesicle elongated, non-bifurcated.

Distribution. Penha, Navegantes, Cabeçudas, Itajaí, Pomerode, Blumenau, Brusque (state of Santa Catarina, SC).

Material examined. EMF=IPP Nr. 1267: Blumenau (SC), 10/VII/1966. Anterior region 1: transverse sections on 10 slides; anterior region 2: sagittal sections on 21 slides; pre-pharyngeal region: transverse sections on 6 slides; pharynx and copulatory apparatus: sagittal sections on 19 slides. EMF=IPP Nr. 1521: Parque Morumbi, São Paulo (SP), 3/VII/1985, O. Françoso, leg. Anterior region 1: transverse sections on 9 slides; copulatory apparatus: sagittal sections on 16 slides. FCB F0072: Cabeçudas (SC), 20/II/2002, F. Carbayo, col. Anterior region 1: sagittal sections on 3 slides. FCB F0079: Navegantes (SC), 21/II/2002, F. Carbayo, col. Preserved in ethanol 70%. FCB F0086: Blumenau (SC), 22/II/2002, F. Carbayo, col. Copulatory apparatus: sagittal sections on 13 slides. EMF=IPP Nr. 1420: Cabeçudas (SC), 20/II/2002, Rejane dos A. de Castro, *leg*. Anterior region 1: transverse sections on 16 slides; copulatory apparatus: sagittal sections on 17 slides. EMF Nr. 192=IPP Nr. 1311: Blumenau (SC), 02/VII/1953. Anterior region 1: sagittal sections on 6 slides; anterior region 2: sagittal sections on 8 slides; anterior region 3: sagittal sections on 7 slides; pre-pharyngeal region: transverse sections on 4 slides; pharynx: sagittal sections on 5 slides; copulatory apparatus: horizontal sections on 10 slides. EMF (=FCB F1006): Blumenau (SC), 17.VII.1966. Anterior region 1: horizontal sections on 3 slides.

External morphology of fixed specimens. *Luteostriata* species of 90 mm in maximum length (Table 1). Body slender (relationship of width: length is 3.6-6.7%); parallel margins; anterior extremity rounded, posterior pointed. Body elliptical in cross-section. Background color of the dorsal surface yellowish with one or three longitudinal stripes arranged in one of the following four color patterns: (a) a median black stripe 1/20th of body width (specimens from Itajaí, Fig. 1); (b) a median black stripe 1/20th of body width, and two lateral ferruginous stripes (specimens from Blumenau, Cabeçudas, Navegantes and Pomerode, Fig. 2); (c) one median black stripe 1/5th of body width (specimens from Brusque, Fig. 3); (d) one median black stripe 1/5th of body width and two lateral ferruginous stripes (specimens from Blumenau, Fig. 4).

Ventral side whitish with the surface of the cephalic glandular area (see below) being slightly darker. The eyes are monolobated, 30-40 µm in diameter, not set in pigment-free areas (halos), encircling the cephalic region. At 8 mm from anterior end of the body, the eye cups extend dorsally as a lateral band being 22% of the body width. Posteriorly this band narrows progressively and is located only at the margin of the body. Sensory pits are simple 28-30 µm deep invaginations; they encircle ventrally the entire cephalic region



Figs 1-4. – *Luteostriata muelleri*. Diagrammatic color pattern of animals from (1) Itajaí, (2) Blumenau and Pomerode, (3) Brusque, and (4) Blumenau.

and extend posteriorly to a distance equivalent to 16% of body length. The width of the creeping sole is 71%-89% of that of the body in the pre-pharyngeal region. Towards the anterior tip, the creeping sole narrows considerably more than the body and disappears close to the tip. Distance of mouth from anterior tip equivalent to 54-72% of the body length; the gonopore, 70-83%.

TABLE 1

Measurements (in mm) of specimens of *Luteostriata muelleri* (Diesing, 1861) comb. nov., living and fixed.

Specimen	**IPP Nr. 1267**	**IPP Nr. 1311**	**IPP Nr. 1420**
Maximum length crawling	?	?	100
Maximum width crawling	?	?	3
Length at rest	?	?	78
Width at rest	?	?	4
L (length after fixation)	49	35	90
W (width after fixation)	2.22	1.71	3.21
height	0.87	0.80	1.46
ratio W : L	4.5%	4.9%	3.6%
M (mouth – anterior body tip)	33.2	20	54
ratio M : L	67.7%	57.1%	54.0%
G (gonopore – anterior body tip)	40.8	26	69.9
ratio G : L	83.3%	74.3%	69.9%

Epidermis and sub-epidermal secretions. The epidermis is ciliated only on the creeping sole. Three types of secretory cells open onto the entire cephalic region: first, cells with fine erythrophilic granular secretions, second, cells with amorphous cyanophilic secretions, and a third type with amorphous xanthophilic secretions (when stained with Cason; also coarse weak cyanophilic granules and erythrophilic cytoplasm when treated with Masson/Goldner stain). Xanthophilic secretions are abundant in the ventral region, constituting the cephalic glands of the musculo-glandular organ. The surface of the epithelium penetrated by the cephalic glands is U-shaped and ca. 3 mm in length (specimen EMF=IPP Nr. 1267). The epithelium traversed by the cephalic glands is 80 µm in height, and that of the adjacent creeping sole is 50 µm high. The ducts of the xanthophilic cells run parallel together in bundles. Their cell bodies are located at least 5 mm behind the anterior end of the body.

Three types of secretory cells open onto the epidermis of the pre-pharyngeal region: cells with erythrophilic granules, cells with cyanophilic granules, and rhabditogen cells. All of these cells increase in number in the dorso-lateral epidermis. Secretory cells with xanthophilic granules also open onto the lateral epidermis. Glandular margin absent.

Cephalic musculature. At approximately 2.5 mm from the anterior end of the body, the longitudinal muscle fibers of the ventral sub-epidermal musculature concentrate progressively in the median region, thus forming a bundle of cephalic retractor muscles, lens-shaped in cross-section. At ca. 2 mm from anterior end of the body, bundles of 2-7 fibers detach from the retractor and continue forwards obliquely to the dorsal surface and the body margins. Subsequently, these bundles separate into single fibers before each fiber attaches to the basement membrane. Some of these fibers traverse to the opposite side of the body before attaching to the basement membrane (Fig. 5).

The large number of secretory cells hampers clear visualization of this mesenchymal muscle system. The most likely muscular organization of the cephalic end is as follows: The most abundant mesenchymal muscle fibers are dorso-ventral, oblique, running from marginal epidermis to that part of the epidermis traversed by the openings of cephalic glands. Other mesenchymal fibers are arranged in four weak layers: a diagonal, a supra-intestinal, a sub-intestinal, and a subneural layer (= under the ventral nerve plate), the latter being the most conspicuous one. At the anterior tip of the body, fibers of this layer anchor onto the basement membrane of the lateral epidermis and onto that of the ventral side.

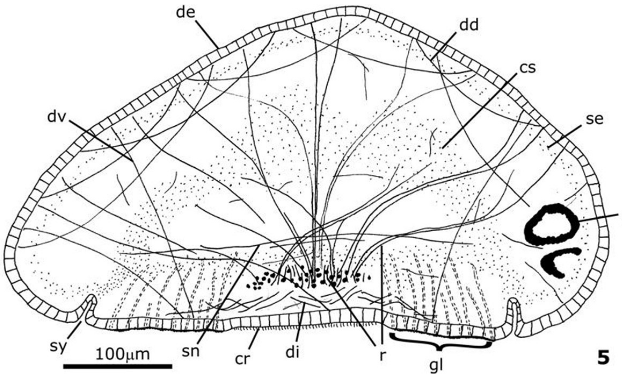


Fig. 5. – *Luteostriata muelleri* (Diesing, 1861) comb. nov. Diagrammatic transverse reconstruction of cephalic region of specimen EMF=IPP Nr. 1267 at 0.2 mm from anterior tip of the body.

Sub-epidermal and mesenchymal musculature at the pre-pharyngeal region. The three typical sub-epidermal layers of the Geoplaninae are present: one of circular fibers –thinner towards body sides–, then a double one with diagonal decussate bundles, and then a layer with longitudinal fibers arranged in bundles (Table 2). The latter is 1.1-1.5 times thicker dorsally than ventrally. SMI, 16.3%-18.9%.

The mesenchymal muscle fibers are extended in various directions. Of these, the dorso-ventral ones, which are partially

TABLE 2

Thickness (in µm) of sub-epidermal musculature in the pre-pharyngeal region of Luteostriata muelleri (Diesing, 1861) comb. nov. The lowest and highest numbers of muscle fibers per bundle are given in parentheses. SMI refers to sub-epidermal musculature thickness relative to body height.

Specimen	IPP Nr. 1267	IPP Nr. 1311
dorsal circular	3 (1-2)	2.2 (1-2)
dorsal diagonal	6 (2-4)	4.3 (2-4)
dorsal longitudinal	102.9 (50-63)	45.6 (48-53)
dorsal total	111.9	52.2
ventral circular	3 (1-2)	2.2 (1-2)
ventral diagonal	8.8 (2-4)	6.5 (2-4)
ventral longitudinal	67.6 (20-25)	34.8 (21-25)
ventral total	79.4	43.5
SMI	17.1%	18.9%
creeping sole : body width	70.6%	88.9%

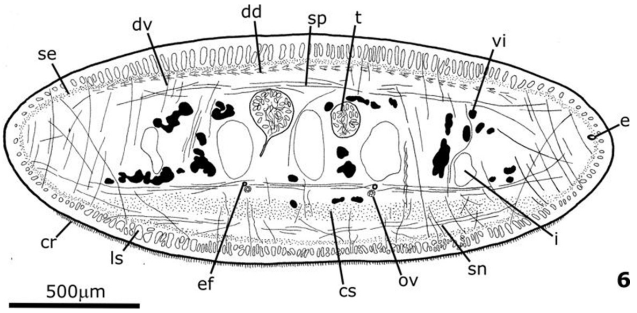


Fig. 6. – *Luteostriata muelleri*. Diagrammatic transverse section of pre-pharyngeal region of specimen EMF=IPP Nr. 1267.

joined in bundles, are the most abundant. Other mesenchymal fibers are arranged in four layers (Fig. 6): (1) well-developed dorsal layer with decussate diagonal fibers (30 µm), (2) supra-intestinal (30 µm), (3) sub-intestinal (12 µm, with part of its fibers joined in bundles), and (4) sub-neural (= under the ventral nerve plate, 38 µm), with transverse muscle fibers. Towards the anterior end, the layers become thinner and eventually are absent. Mesenchymal longitudinal musculature absent.

Digestive system. Mouth at the end of the first-third of the pharyngeal pocket. Pharynx bell-shaped (Fig. 7), with the dorsal insertion at the level with the mouth, occupying most of the pharyngeal pocket. Esophagus absent. Pharyngeal pocket: musculature composed of a one-fiber-thick sub-epithelial layer with circular fibers. Outer pharyngeal musculature composed of a longitudinal layer (2 µm), followed by a circular one (20-25 µm) with some intermingled longitudinal fibers at its innermost region. Inner pharyngeal musculature composed of a sub-epithelial circular layer (12 µm) followed by a longitudinal one (5 µm).

Male reproductive system. Testes rounded, approximately 250 µm in diameter, dorsal in position, located under the supra-intestinal muscle layer and between the intestinal branches (Fig. 6). Testis follicles are arranged in one irregular row on each side of the body, extending from the ovaries to the pharynx. The most anterior and posterior testes are located at a distance equal to 25% and 66% of body length in relation to anterior end, respectively (specimen EMF=IPP Nr. 1267).

The efferent ducts run between the fibers of the sub-intestinal muscle layer or just above this layer. These ducts recurve before opening laterally into the anterior region of the prostatic vesicle (Figs 8-9). The prostatic vesicle is an elongated sinuous (~10 x as long as wide) and non-bifurcated cavity with folded walls. The distal portion of the prostatic vesicle penetrates the common muscle coat and the penis bulb and continues as an ejaculatory duct. The latter opens into the anterior region of the male atrium, which is an irregularly folded cavity. In specimen EMF=IPP Nr. 1267, there is a penis-shaped fold in the anterior region of the atrium (asterisk in Fig. 9).

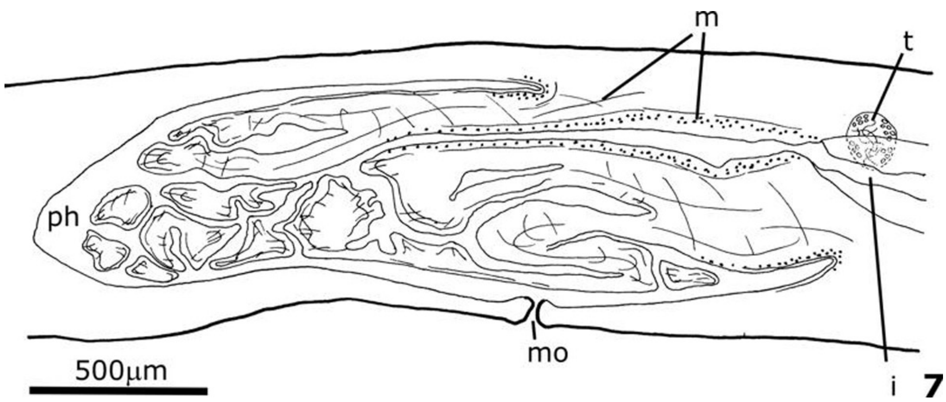


Fig. 7. – *Luteostriata muelleri*. Drawing of a sagittal section of the pharynx of specimen EMF Nr. 192=IPP Nr. 1311.

The efferent ducts are lined with a ciliated, cuboidal epithelium. The distal portion of these ducts receives erythrophilic granular secretions. The ducts are surrounded by circular muscle fibers (10 µm). The prostatic vesicle is composed of a columnar ciliated epithelium that becomes cuboidal at its distal portion. Two types of secretory cells traverse the epithelium of the prostatic vesicle: a type with erythrophilic granules, and another type with xanthophilic granules that is most abundant proximally and scarce distally. The prostatic vesicle is surrounded by circular muscle fibers (37 µm). The ejaculatory duct has a cuboidal, ciliated epithelium, and is surrounded by a layer of circular muscle fibers (6 µm). The male atrium has a columnar, non-ciliated epithelium, the proximal half of which is apically lobed. Epithelium of the ventral side of the atrium erythrophilic. Cyanophilic granular secretory cells and erythrophilic granular secretory cells open through the entire epithelium of the atrium. The musculature of the atrium consists of a sub-epithelial layer with circular fibers (5-15 µm; thinner proximally) followed by a thin layer with longitudinal fibers (5 µm). In the posterior region of the atrium, the fibers of these layers are partially intermingled.

Female reproductive system. Ovaries elongated (600 µm x 100 µm), ventral in position, between the sub-intestinal muscle layer and the ventral nerve plate, lying at a distance of 25% of body length from the anterior body end. The ovovitelline ducts arise from the medium dorsal side of ovaries and run posteriorly between the sub-intestinal muscle layer and the ventral nerve plate. The ducts join dorsally to the female atrium to form a common oviduct (Figs 8-9). The common glandular ovovitelline duct, 5x longer than wide, curves downwards to open into an obliquely-oriented diverticulum (vagina) of the funnel-shaped female atrium. Percentage, length of female atrium: length of male atrium, 15-36%.

The ovovitelline ducts are lined with a ciliated, cuboidal epithelium; shell glands open into the distal portions of the ducts, which are covered with a layer of circular muscle fibers (5 µm). The common glandular ovovitelline duct has a columnar, ciliated epithelium, and is also covered with a layer of circular muscle fibers (4 µm). Diverticulum of the female atrium or vagina lined with columnar epithelium and surrounded by a layer of circular muscle fibers (7 µm). This vagina is penetrated by the openings of granular, erythrophilic glands. Female atrium lined with a non-ciliated columnar epithelium and receiving the openings of erythrophilic granular glands as well as granular cyanophilic ones. Female atrium surrounded by scarce longitudinal muscle fibers that are continuous with the common muscle coat around the entire copulatory complex. This common muscle coat consists of a layer of longitudinal and diagonal fibers, thicker in the region of the male atrium (18 µm) than in the region of the female atrium (8 µm) (Figs 8-9).

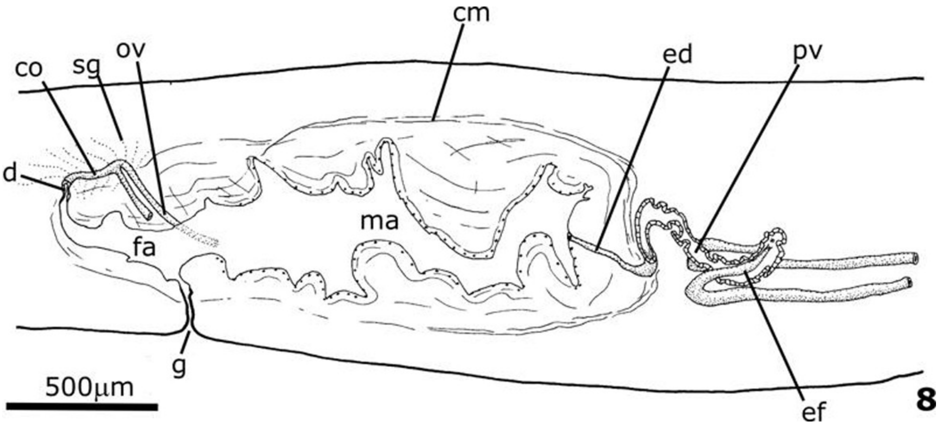


Fig. 8. – *Luteostriata muelleri*. Diagrammatic sagittal reconstruction of the copulatory apparatus of specimen FCB F0086**.**

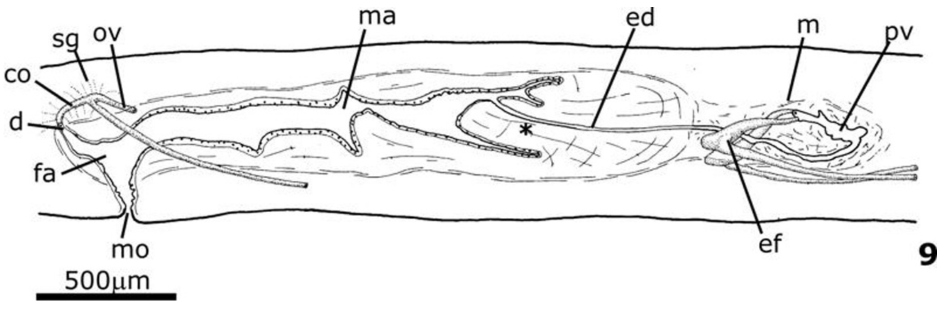


Fig. 9. – *Luteostriata muelleri*. Diagrammatic sagittal reconstruction of the copulatory apparatus of specimen EMF=IPP Nr. 1267. Asterisk indicates a penis-shaped fold in male atrium.

Luteostriata abundans (Graff, 1899) comb. nov.
Figs 10-11

Diagnosis. Dorsal surface with seven black stripes. Prostatic vesicle elongated, non-bifurcated.

Distribution. Nova Petrópolis (new record), Taquara, Parobé (new record), Tupandi, Glorinha, Poço das Antas, São Sebastião do Caí, Campo Bom, Novo Hamburgo, São Leopoldo, Salvador do Sul, Porto Alegre, Barra do Ribeiro (new record) (state of Rio Grande do Sul, RS).

Ecology. Species common in urban environments. In natural forests only seen once by the author (municipality of Barra do Ribeiro, RS). One specimen found in an urban garden in Porto Alegre (RS), 19-V-1999 in a nest of Termitidae, under a fallen log, probably feeding on the termites. Another specimen was found feeding on a woodlouse *Atlantoscia floridana* (van Name, 1940) under wooden boards in a rural environment at Novo Hamburgo (RS), 7-XI-2004.

Material examined. MZU PL. 00061: São Leopoldo (RS), 19/VIII/1997, W. Hilier and L. Dornelles, *leg*. Anterior region 1: transverse sections on 16 slides; anterior region 2: sagittal sections on 16 slides; pre-pharyngeal region: transverse sections on 8 slides; pharynx: sagittal sections on 13 slides; copulatory apparatus: horizontal sections on 37 slides. MZU PL. 00064: Novo Hamburgo (RS), 21/X/1997, M. Cardoso, *leg*. Anterior region 1: sagittal sections on 9 slides; anterior region 2: sagittal sections on 12 slides; anterior region 3: sagittal sections on 17 slides; pre-pharyngeal region: transverse sections on 4 slides; pharynx: sagittal sections on 14 slides; copulatory apparatus: sagittal sections on 14 slides. MZU PL.00068: São Leopoldo (RS), 25/VIII/1998, F. Carbayo and M. Urien Herrero, *leg*. Anterior region 1: sagittal sections on 8 slides; anterior region 2: sagittal sections on 17 slides; anterior region 3: sagittal sections on 8 slides; pre-pharyngeal region: transverse sections on 10 slides; pharynx: sagittal sections on 26 slides; copulatory apparatus: sagittal sections on 49 slides. FCB F0225: Parobé (RS), 27/III/2004, F. Carbayo, col. Anterior region 1: horizontal sections on 3 slides. FCB F0372: Taquara (RS), 10/VII/2004, F. Carbayo, col. Anterior region 1: sagittal sections on 6 slides. FCB F0391: Nova Petrópolis (RS), 05/VI/2004, F. Megiolaro, leg. Anterior region 1: horizontal sections on 3 slides.

Morphological notes. Anterior region of the body ventrally slightly concave, more accentuated when creeping. Sensory pits simple invaginations 15 µm deep, ventrally encircling the entire anterior tip and extending posteriorly until a distance equivalent to 15-20% of body length. Epidermis only ciliated on the creeping sole; the first 4-6 millimeters of the ventral anterior region are laterally gray-yellowish, and slightly protuberant, delimiting the glandular surface of the cephalic glands of the musculo-glandular organ. This epdermis is 20 µm high, while that of the rest of the ventral cephalic region is 13 µm high. The cell glands are of three types: 1) abundant cells with coarse xanthophilic granules, 2), cells with fine erythrophilic granules, and 3), scarce, with fine cyanophilic granules. Ducts of erythrophilic cells traverse the epidermis as bundles. At 7-10 mm from the anterior tip, the layer with longitudinal sub-epidermal muscle fibers is ventrally 1.5 to 2.9 times thicker than dorsally. More anteriorly, this muscle layer, already modified as the retractor (Figs 10-11), is 1.7 times thicker. The retractor is similar to that of *L. muelleri*; however, fibers traversing to the opposite side were not found.

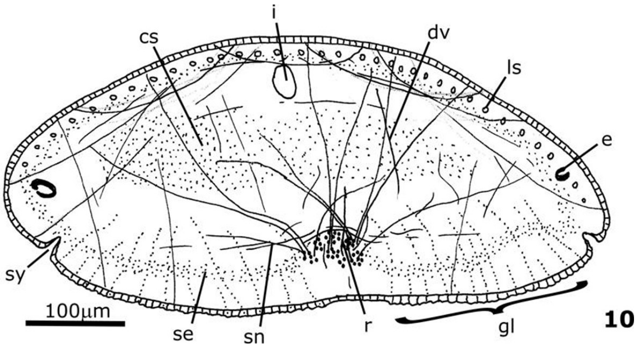


Fig. 10. – *Luteostriata abundans*. Diagrammatic transverse reconstruction of the cephalic region at 0.2 mm from anterior end of specimen MZU PL.00061.

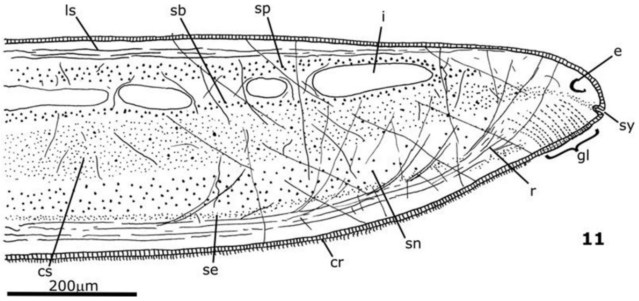


Fig. 11. – *Luteostriata abundans*. Diagrammatic sagittal section of cephalic region of specimen MZU PL.00064.

Luteostriata caissara (Froehlich, 1955) comb. nov

Diagnosis. Dorsal surface with five evenly-distributed dark longitudinal stripes. Prostatic vesicle elongated, bifurcated, and intricately folded, with bifurcations embracing the pharynx.

Distribution. Teresópolis, Barreira, Gávea Pequena (state of Rio de Janeiro, RJ), Ubatuba, Ribeirão Pires, Itanhaém (state of São Paulo, SP).

Material examined. See Carbayo (2006). Specimens studied are deposited in the MZU (Museu de Zoologia da

Morphological notes. Carbayo (2006) incorrectly described the organization of the muscle layers underlying the inner pharyngeal epithelium. The arrangement is as follows: immediately beneath the epithelium lies a layer comprised of circular muscle fibers (30 µm), followed by a layer of longitudinal fibers (10-15 µm).

Luteostriata ceciliae (Froehlich & Leal-Zanchet, 2003) comb. nov.

Figs 12-14

Diagnosis. Dorsal surface with five longitudinal black stripes; wide marginal zone without stripes. Efferent ducts branched, each branch opening into the proximal half of the prostatic vesicle, the latter being bifurcated.

Distribution. São Francisco de Paula (state of Rio Grande do Sul, RS).

Material examined. São Francisco de Paula. Paratype: MZUSP PL. 151: 25/XI/1998, S.A. Souza, *leg*. Anterior region 1: horizontal sections on 5 slides; anterior region 2: sagittal sections on 18 slides; anterior region 3: sagittal sections on 18 slides; pre-pharyngeal region: transverse sections on 16 slides; pharynx: sagittal sections on 17 slides; copulatory apparatus: sagittal sections on 18 slides. Paratype MZU PL. 00029: 16/XII/1998, M. Cardoso, *leg*. Anterior region 1: transverse sections on 15 slides; anterior region 2: horizontal sections on 4 slides; anterior region 3: sagittal sections 16 slides; pre-pharyngeal region: transverse sections on 7 slides; pharynx: horizontal sections on 6 slides; copulatory apparatus: horizontal sections on 33 slides. Paratype MZU PL. 00027: 8/VI/1998, L. dos Santos Teixeira, *leg*. Anterior region 1: sagittal sections on 7 slides. Paratype MZU PL. 00028: 31/VII/1998, M. Cardoso, *leg*. Anterior region 1: sagittal sections on 9 slides.

Morphological notes. Sensory pits are simple invaginations 20 µm deep; ventrally encircling the entire cephalic region in a single row and extending posteriorly at a distance equivalent to 17% of the body length. Epidermis ciliated only on the creeping sole. Dorsal epidermis 10 µm high, 15 µm on the medio-ventral body region. Epidermis 10, 15 and 22 µm in height on the dorsal, the medio-ventral, and the ventral glandular region of the musculo-glandular organ, respectively. The latter is 2.5-3 mm in length (Fig. 12), and its epidermal glandular surface is orange-brownish and penetrated by the openings of two types of secretory cells: (1) cells with fine erythrophilic granules, and (2) cells with coarse weak cyanophilic granules. The necks of the erythrophilic glands run joined. At 2 mm from the anterior tip of the body, the ventral longitudinal sub-epidermal musculature is modified as a retractor, 1.6 times thicker than at 6 mm from the tip (specimen MZU PL. 00029), where it is 30 µm thick. Retractor (Figs 13-14) similar to that of *L. muelleri*.

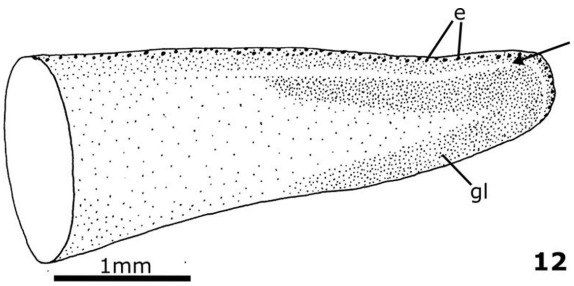


Fig. 12. – *Luteostriata ceciliae*. Perspective drawing of the cephalic region of specimen IPP Nr. 346d mainly showing the ventral surface. Arrow indicates sensory border.

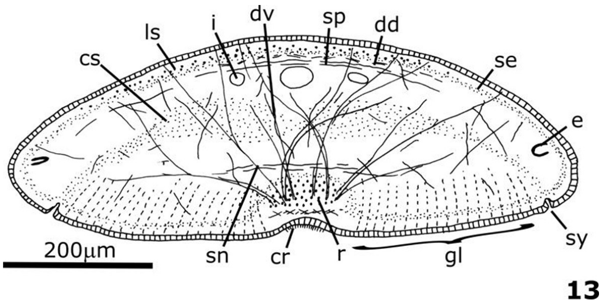


Fig. 13. – *Luteostriata ceciliae*. Diagrammatic transverse reconstruction of the cephalic region at 0.7 mm from anterior end of specimen MZU PL. 00029.

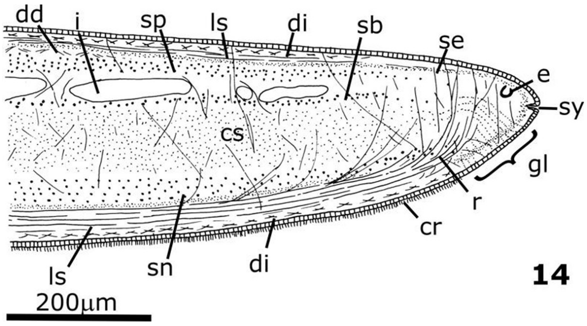


Fig. 14. – *Luteostriata ceciliae*. Diagrammatic sagittal section of cephalic region of specimen MZU PL. 00027.

Luteostriata ernesti (Leal-Zanchet & Froehlich, 2006) comb. nov.

Figs 15-16

Diagnosis. Dorsal surface with five evenly-distributed longitudinal black stripes, the median one being the thinnest. Prostatic vesicle elongated and non-bifurcated.

Distribution. Pirassununga, Valinhos, Jundiaí, São Paulo, Mogi das Cruzes, Ibiúna, Ribeirão Pires (state of São Paulo, SP), Curitiba (state of Paraná, PR), São Francisco de Paula (state of Rio Grande do Sul, RS).

Material examined. MZUSP PL. 174: São Francisco de Paula (RS), 5/XII/2002, R. A. de Castro, *leg*. Anterior region 1: transverse sections on 4 slides. EMF=IPP Nr. 1289: Ibiúna (SP), 26/VII/1965. Anterior region 1: sagittal sections on 2 slides; copulatory apparatus: sagittal sections on 9 slides. MZU PL.00047: São Francisco de Paula (RS), 25/IX/1998, F. Carbayo, *leg*. Anterior region 1: sagittal sections on 3 slides; pre-pharyngeal region: transverse sections on 9 slides; pharynx: sagittal sections on 13 slides; copulatory apparatus: sagittal sections on 17 slides. EMF Nr. 927: Serra do Japi, Jundiaí (SP), 22/VI/1996, C. F. Rocha, *leg*. Anterior region 1: transverse sections on 5 slides.

Morphological notes. Ventral side concave, more accentuated when creeping. Sensory pits are simple invaginations 20 µm deep, ventrally-encircling the entire cephalic region and extending posteriorly to a distance equivalent to 8% of body length. Epidermis only ciliated on the creeping sole. First 4-6 millimeters of the ventral cephalic tip slightly darker, denoting the glandular region. Epidermis in this region 22 µm high, 7 µm higher than on the remainder of ventral cephalic region. Dorsal epidermis 12 µm high. Cephalic glands of two types: cells with xanthophilic granules, and cells with coarse granules with weak affinity to the cyanophilic stain. Distal portion of xanthophilic cellular ducts run joined in bundles. At 2.5 mm from the anterior tip, ventral layer with sub-epidermal longitudinal muscle fibers is 25 µm thick. At this point, the dorsal sub-epidermal longitudinal layer is 20 µm. More anteriorly, the dorsal layer is modified as the retractor muscle, 25 µm thick (Figs 15-16). Arrangement of its fibers is similar to that in *L. muelleri*.

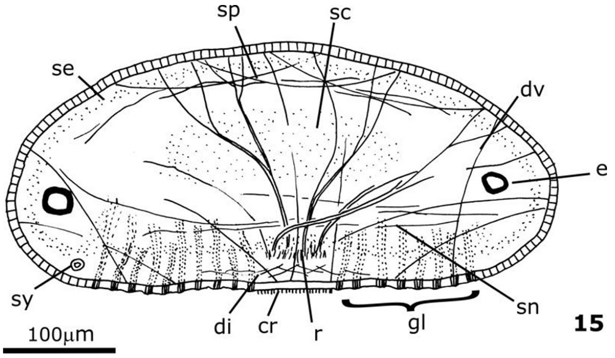


Fig. 15. – *Luteostriata ernesti*. Diagrammatic transverse reconstruction of cephalic region of specimen EMF=IPP Nr. 1521 at 0.15 mm from anterior tip of the body.

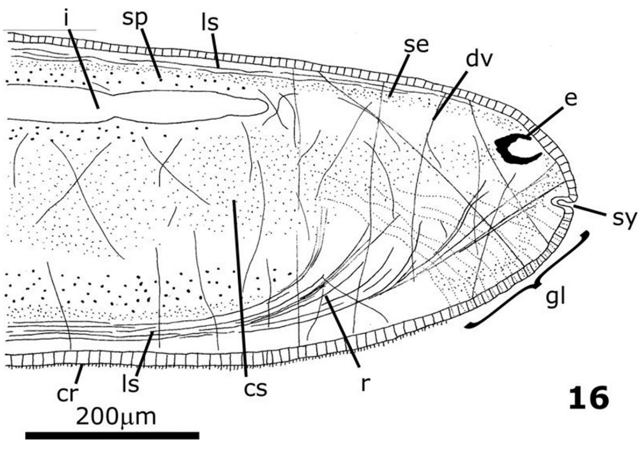


Fig. 16. – *Luteostriata ernesti*. Diagrammatic sagittal section of cephalic region of specimen EMF=IPP Nr. 1289.

Luteostriata fita (Froehlich, 1959) comb. nov.

Diagnosis. Very slender body. Dorsal surface with four longitudinal dark stripes plus a fifth one in the cephalic region. Dorsal cephalic epidermis ciliated. Pharyngeal pocket twice the length of the pharynx. Efferent ducts open into the posterior region of the prostatic vesicle, the latter being very elongated and non-bifurcated.

Distribution. Blumenau (state of Santa Catarina, SC).

Material examined. See Carbayo (2006).

Luteostriata graffi (Leal-Zanchet & Froehlich, 2006) comb. nov.

Figs 17-18

Diagnosis. Dorsal surface with five black stripes: a median one, two lateral and two sub-marginal stripes; the lateral stripes the widest, being about 1/5 of the body width. Female atrium as long as the male atrium.

Distribution. Praia Grande (state of Santa Catarina, SC), São Francisco de Paula, Três Coroas, Taquara, Parobé (new record), Morro Reuter (new record), Salvador do Sul, São Leopoldo (state of Rio Grande do Sul, RS).

Material examined. MZUSP PL. 176: São Francisco de Paula (RS), 25/XI/1998, F. Carbayo, *leg*. Anterior region 1: sagittal sections on 4 slides; anterior region 2: sagittal sections on 12 slides; pre-pharyngeal region: transverse sections on 5 slides. Paratype MZU PL.00052: São Francisco de Paula (RS), 11/XII/1997, M. Cardoso, *leg*. Anterior region 1: sagittal sections on 4 slides; anterior region 2: sagittal sections on 10 slides; pre-pharyngeal region: transverse sections on 16 slides. Paratype MZU PL.00058: Salvador do Sul (RS), 26/VII/2000, A. M. Leal-Zanchet, *leg*. Anterior region 1: transverse sections on 19 slides. FCB F0204: Parobé (RS), F. Carbayo, col. Anterior region 1: horizontal sections on 5 slides;

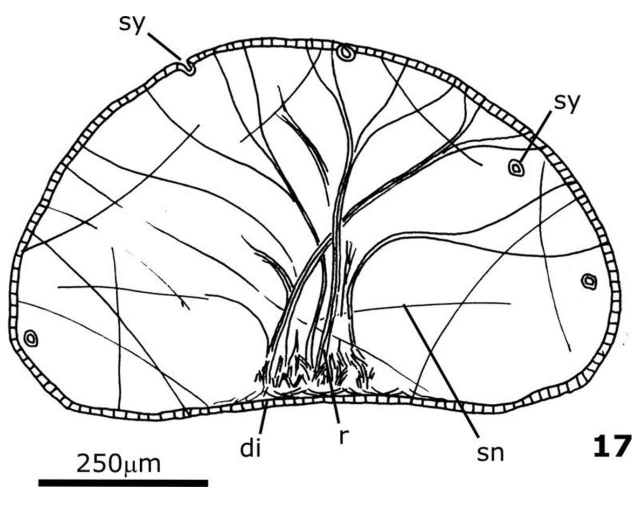


Fig. 17. – *Luteostriata graffi*. Diagrammatic horizontal reconstruction of cephalic region at 0.5 mm from anterior end of the body of specimen FCB F0204.

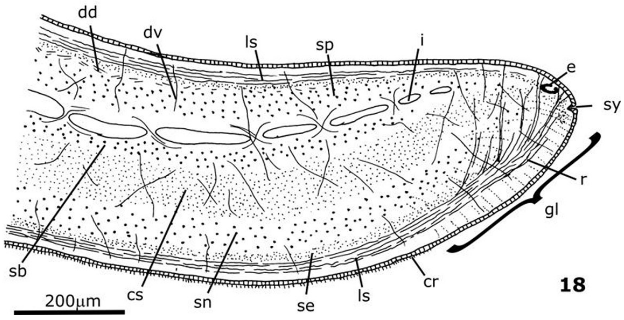


Fig. 18. – *Luteostriata graffi*. Diagrammatic sagittal section of cephalic region of specimen MZUSP PL. 176.

anterior region 2: horizontal sections on 3 slides; pre-pharyngeal region: transverse sections on 4 slides; pharynx: sagittal sections on 4 slides; copulatory apparatus: sagittal sections on 4 slides. FCB F0467: Morro Reuter (RS), F. Carbayo, col. Copulatory apparatus: sagittal sections on 5 slides.

Morphological notes. Sensory pits are simple invaginations, 20 µm deep, ventrally-encircling the entire cephalic region and extending posteriorly to a distance equivalent to 21-25% of body length. Epidermis only ciliated on the creeping sole. The epidermis is 12 µm high dorsally, 15 µm ventrally. Glandular region in the ventral cephalic end 2-3 mm in length. In fixed animals, the surface of the cephalic glands is slightly protuberant. The organ is formed by two types of secretory cells: (1) abundant cells with erythrophilic granules and necks running joined, and (2) cells with fine pink granules (stained with Cason and Masson-Goldner). At the point where the epidermis is traversed by the openings of these secretory cells, it is 20 µm in height. In the first 4 millimeters of the body, the organization of the ventral longitudinal sub-epidermal musculature is different from that in the rest of the body. At 2 mm from the anterior tip, this layer is modified to form a retractor muscle similar to that of *L. muelleri*, 1.7 times thicker than at the pharyngeal region, and traversed by some dorso-ventrally-running mesenchymal fibers (Figs 17-18).

DISCUSSION

Luteostriata nov. gen. differs from all other geoplaninid genera in the following combination of features: it has a slender body with a slightly rounded, not rolled cephalic region; cephalic musculo-glandular organ present, consisting of glands crossing a U-shaped glandular ventral surface; lens-shaped retractor muscle in cross-section; close to the anterior end of the body, the fibers of the retractor detach from it and are sunken into the mesenchyma, continuing forwards obliquely to the dorsal surface and the body margins before attaching to the basement membrane; transversal sub-neural muscle fibers and those of the retractor do not intermingle.

Below we discuss the differences between the new genus and the geoplaninid genera that also have a cephalic musculo-glandular organ, viz., *Cephaloflexa* Carbayo & Leal-Zanchet, 2003, *Choeradoplana* Graff, 1896, *Issoca* Froehlich, 1955 and *Supramontana* Carbayo & Leal-Zanchet, 2003 (Tab. 3). The retractor muscle of the organs of these genera is also derived from the ventral longitudinal sub-epidermal musculature.

Luteostriata nov. gen. is distinguished from *Cephaloflexa* and *Choeradoplana* in that, in the latter two genera (a) the cephalic region is backwardly-rolled; (b) eyes and sensory pits are absent at the anterior tip of the body; (c) the fibers of the retractor muscle run parallel to the sagittal plane; and (d) the sub-neural mesenchymal muscle layer is present only in the cephalic region. Furthermore, in *Cephaloflexa* (e) the cephalic region is very narrow; and (f) glands associated with the retractor muscle are absent, while in *Choeradoplana* (g) the cephalic region is laterally expanded; (h) and the glandular surface of the cephalic musculo-glandular organ has two cushion-like organs; (i) the retractor is deltoid in cross-section; (j) in the cephalic region a dense muscle net ("Muskelgeflecht"), is present and formed by fibers of the supra-intestinal mesenchymal transverse muscle layer and by those of the dorso-ventral mesenchymal muscle system; (k) in the cephalic region cellular bodies of rhabditogen cells lie between the retractor muscle and the epidermis; and (l) part of the sub-epidermal longitudinal muscle layer is sunken along the entire body (Tab. 3).

Luteostriata gen. nov. is also different from in *Issoca* in that, in the latter (a) the cephalic region is spoon-shaped; (b) the glandular surface of the cephalic musculo-glandular organ is lunate; (c) the retractor muscle is rounded in cross section; (d) the sub-neural mesenchymal muscle layer is intermingled with retractor.

From *Supramontana*, *Luteostriata* gen. nov. differs in that the former genus (a) possesses a relatively wide body with a rounded anterior end; (b) cephalic glands associated with the retractor muscle are absent; (c) the retractor is irregularly lenticular; (d) cellular bodies of rhabditogen cells lie between the retractor muscle and the epidermis in the cephalic region; and (e) part of the sub-epidermal longitudinal muscle layer is sunken along entire body.

The cephalic regions of *Notogynaphallia arturi* and *N. pseudoceciliae*, both assigned to group 2 (Froehlich & Leal-Zanchet 2003), should be investigated in order to check whether they match the diagnostic features of the new genus.

Some observations on feeding behavior may help to further understand the function of the cephalic musculo-glandular organ. Froehlich (1955a) suggested a role for the organ of *Issoca rezendei* hunting prey. The action of the retractor of this organ could provide a sucker-like function to the cephalic ventral surface of the flatworm by increasing the concavity of this surface. This sucker action, combined with the putative adhesive nature of the abundant erythrophilic granular secretions of the cephalic glands (Froehlich, 1955), could facilitate the capture of prey. E. M. Froehlich (unpublished) observed in laboratory conditions that *Issoca rezendei* is able to attack woodlice (Crustacea: Isopoda), a behavior rarely observed in land planarian species. During this attack the animal swiftly throws its anterior body region onto the prey, with its ventral cephalic surface directed forwards and the cephalic region curved dorsally (pers. obs.).

TABLE 3
Diagnostic features of the five genera of Geoplaninae provided with cephalic retractor muscle.

Feature	***Cephaloflexa***	***Choeradoplana***	***Supramontana***	***Issoca***	***Luteostriata* nov. gen.**
body	slender	slender	wide	slender	slender
cephalic shape	very narrow, rolled backwards	expanded, rolled upwards	rounded	spoon-shaped	slightly rounded
eyes at anterior tip of the body	no	no	yes	yes	yes
sensory pits at the anterior tip of the body	no	no	yes	yes	yes
cephalic glands associated with retractor muscle	no	yes	no	yes	yes
shape of epidermal surface crossed by cephalic glands	-	two elongate cushions	-	lunate	U-shaped
cross sectioned retractor	lenticulate	deltoid	irregularly lenticulate	rounded	lenticulate
retractor muscle fiber orientation	parallel to sagittal plane	parallel to sagittal plane	towards body margins	towards body margins	towards dorsal surface and body margins, some fibers cross the sagittal plane
sub-neural mesenchymal muscle layer	only in cephalic region	only in cephalic region	along entire body	along entire body	along entire body
sub-neural mesenchymal muscle layer intermingled with retractor	no	no	no	yes	no
Muskelgeflecht	no	yes	no	no	no
cellular bodies of rhabditogen cells between retractor and epidermis	no	yes	yes	no	no
part of sub-epidermal longitudinal muscle layer sunken along entire body	no	yes	yes	no	no

If the attack succeeds, the isopod is first held on the glandular surface of the organ, hindering the woodlouse's escape, which is then manipulated by the flatworm for feeding.

Likewise, *L. abundans* (see Prasniski & Leal-Zanchet, 2009), *L. caissara* (Bresslau, in Riester, 1938; Froehlich, 1955b), *L. ernesti* (see above) also feed on woodlice. Under laboratory conditions, *L. ernesti* attacks such prey in a manner similar to *I. rezendei* (pers. obs.).

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Exotic freshwater planarians currently known from Japan

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ABSTRACT. Biogeographical and taxonomic information on the four non-indigenous freshwater planarians of Japan is reviewed, viz. *Dugesia austroasiatica* Kawakatsu, 1985, *Girardia tigrina* (Girard, 1850), *G. dorotocephala* (Woodworth, 1897), and *Rhodax evelinae*? Marcus, 1947. The occurrence of *Girardia dorotocephala* in Japan is unequivocally demonstrated. New karyological data are presented for populations of *D. austroasiatica* (chromosome complement: 2x=16, 3x=24), *G. tigrina* (2x=16, 3x=24), and *G. dorotocephala* (2x=16). The following factors may have facilitated the introduction and subsequent geographical spread of exotic freshwater triclads in Japan: popularization of domestic tropical fish cultures, and culture of exotic aquatic animals for food.

KEY WORDS: Tricladida, freshwater, exotic, introduced, Japan

INTRODUCTION

The purpose of the present paper is to review the scattered information available in the literature on non-indigenous freshwater planarians in Japan, and to present new karyological and taxonomic data on some of these species. Furthermore, we discuss possible factors that may have facilitated the introduction and subsequent geographical spread of exotic freshwater triclads in Japan.

MATERIALS AND METHODS

Samples of four species of non-indigenous freshwater planarians were obtained from more than 20 stations (Fig. 1). Collection data for each station will be given below under the species accounts. Both living and preserved specimens were used for the morphological observations. External characters used for tentative identification of the species are shown in Kawakatsu et al. (2009, fig. 2; see also Kawakatsu et al. 2007b, c). For the preservation of planarians 70% ethanol was used in most cases, while Bouin's fluid was used in some cases. Serial sections were made at intervals of 7-8µm and were stained with Delafield's haematoxylin and erythrosin or Mallory-Cason. Reconstructions of the copulatory apparatus were obtained using a camera lucida attached to a compound microscope. Some of the histological material examined for this paper is deposited in the Zoological Museum Amsterdam (ZMA). Preparations of chromosomes were obtained by the squash method of Yamamoto: (1) animals were cut transversally at the basal level of the auricles and were kept for 2 days in a covered Petri dish filled with tap water; (2) the regenerating tissues were treated with a solution of 10^{-6} M colchicine for about 30 minutes; (3) tissues were soaked in 45% acetic acid prior to staining with aceto-orcein (cf. Oki et al. 1980).

SPECIES ACCOUNTS

Dugesia austroasiatica Kawakatsu, 1985

Material examined and distribution: Honshû: Stations 1, 3, 8, 12, 13, and 14 (the only naturalized population). Kyûshû: Station 7 (Fig.1).

Morphology. A rather small, slender, and pigmented species (ca. 12 mm long and 1.5 mm wide in large, sexually mature specimens) inhabiting warm waters. Head subtriangular with a pair of bluntly pointed auricles; with two eyes, each surrounded by a narrow non-pigmented ocular area; the distance between the pigment cups is slightly smaller than one-third of the width of the head at the level of eyes (Fig. 2) (cf. Kawakatsu et al. 2009).

Karyology. Idiograms of *D. austroasiatica* from the Saga locality (St. 7) and the Chiba (Isumi) locality (St. 8) were reported earlier (Fig. 3; cf. Kawakatsu et al. 1986, 1993a). Five new idiograms of populations from 3 localities (Stations 12, 13, and 14) are presented in Fig. 4.

Discussion. For additional data, including locality information, see Hirao et al. (1970) and Kawakatsu et al. 1985 (for stations 1 and 3); Kawakatsu et al. (1986b) (station 7); Kawakatsuet al. (1993a) (station 8); Kawakatsu et al. (2007c) (stations 12 and 13); Kawakatsu et al. (2007b, c) (station 14).

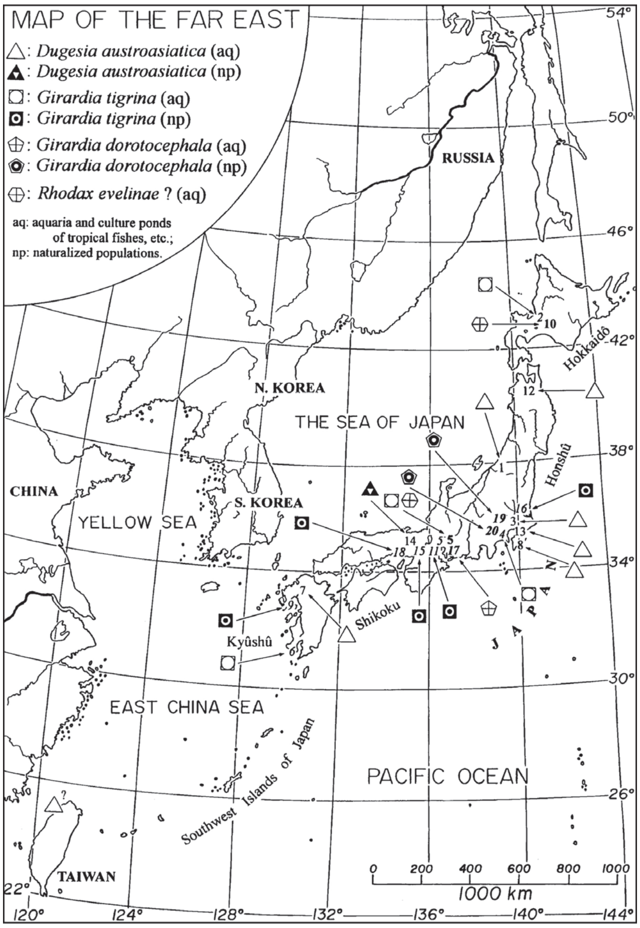

Fig. 1. – Map of the Japanese Islands, showing the geographical distribution of 4 species of exotic freshwater planarians from 20 stations:
1. Aquarium in Nîgata City; 2. Aquarium in Sapporo City; 3. Aquarium of Inokashira Park, Mitaka City; 4. Aquarium in Yokohama City; 5. Aquarium in Nagoya City; 6. Culture ponds of West Australian crayfish in Ibusuki City; 7. Culture ponds of tropical fishes in Saga City; 8. Domestic aquaria in Isumi City; 9. Urakami-gawa River, Nagasaki City; 10. An aquarium in Sapporo City; 11. Lake Biwa-ko in Moriyama City; 12. Domestic aquarium in Aomori City; 13. Domestic aquarium in Tôkyô; 14. Mizoro-gaike Pond in Kyôto City; 15. Kamo-gawa River in Kyôto City; 16. Shallow waters in lowland areas in the vicinity of Mitsukaidô City; 17. Tropical fish cultre tank of the Hekinan Sea Side Aquarium in Hekinan City; 18. Muko-gawa River in Amagasaki City; 19. Lower stream of the Asa-kawa River, a tributary of the Tama-gawa River, Hino City (3 localities); 20. Midstream of the Sagami-gawa River and its tributaries, Sagamihara City (3 localities).

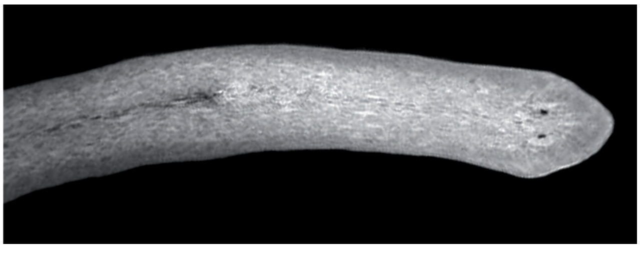

Fig. 2. – External features of *Dugesia austroasiatica.*

Girardia tigrina (Girard, 1850)

Material examined and distribution: Hokkaidô: Station 2. Honshû: Stations 4, 5, 11, 15, 16, and18. Kyûshû: Stations 6 and 9 (Fig. 1).

Naturalized populations concern Stations 11, 15, 16, 18, and 9. Recently, a new naturalized population of *G. tigrina* was found in Kagamihara City (Gifu Pref.), about 50 km N of Station 5.

Morphology. Living, asexual specimens are approximately 10 mm long and 1 mm wide. Head equilateral-triangular, with a pair of broad, short auricles. Two eyes are conspicuous, each surrounded by a large, non-pigmented ocular area. The distance between the eyes is 1/4th-1/5th of the width of the head at level of the auricles. Ground colour of the dorsal surface pale brown with numerous whitish and yellowish pigment spots. Small masses of irregularly arranged blackish and yellowish brown pigment granules are conspicuous on the surface of the pharynx (Fig. 5).

Since sexual specimens of this *Girardia* species were not available, its tentative identification is based only on external features (cf. Kawakatsu et al. 2009). However, external morphology of the *Girardia* species from Japan is very similar to that of specimens of *G. tigrina* from the U.S.A., Mexico, Brazil, and Uruguay (cf. Kawakatsu et al. 1981b, 1982, 1983, 1986a, 1992).

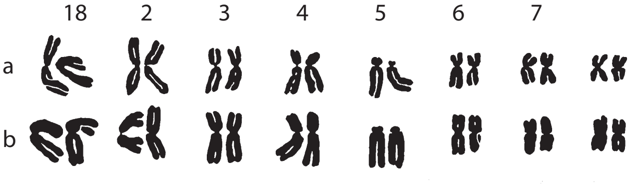

Fig. 3. – *Dugesia austroasiatica*. Two idiograms of population from Station 8, as reported earlier (after Kawakatsu et al., 1993a). a and b: 2x = 16, with a karyotype of 2m + 2m + 2m + 2m/2sm + 2st + 2m + 2m +2m.

Fig. 4. – *Dugesia austroasiatica*. Five new idiograms of populations from Stations 12 (b and b'), 13 (c and c'), and 14 (d); b and b' and c and c' found in somatic cells of a single specimen, respectively.
b and b': 2x = 16, with a karyotype of 2m + 2m + 2m + 2m/2sm + 2st + 2m + 2m +2m. c: 2x-1 + 2LB = 16-1 + 2LB, with a karyotype of 2m + 2m + 2m + 2m + 1st + 2m + 2m + 2m + 2LB. c': 3x=24, with a karyotype of 3m + 3m + 3m + 3m + 3st + 3m + 3m +3 m. d: 2x=16, with a karyotype of 2m+2m+2m+2m+2st+2m+2m+2m.

Karyology. Three new idiograms of *G. tigrina* from Lake Biwa-ko (St. 11) are given in Fig. 6. The diploid karyotype is 2x=16, with 8 pairs of metacentric chromosomes. Triploid karyotypes also occurred. Usually, diploid and triploid karyotypes were found in different specimens.

Judging from the idiograms of *G. tigrina* from Japan published earlier, the 6th (or the 7th) pair of chromosomes were submetacentric in some cases, in contrast to the results published in the present paper (cf. Kawakatsu et al. 1985, 1993b, 2007b, 2008; Tamura et al. 1985; Oki et al. 1995; Chinone et al. 2008). A similar situation was encountered in Brazilian *G. tigrina* (Kawakatsu et al. 1981b, 1986; see also Benya et al. 2007). Italian *G. tigrina* has a karyotype of 7 pairs of metacentric chromosomes and one pair of submetacentric chromosomes (Fig. 7) (Benazzi 1970).

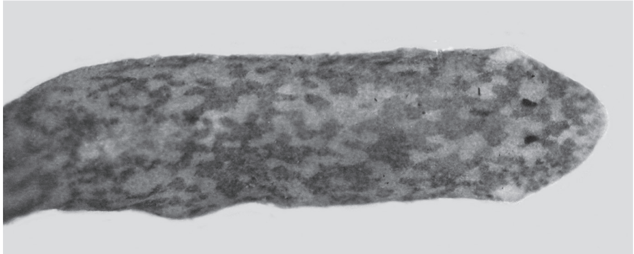

Fig. 5. – External features of *Girardia tigrina*.

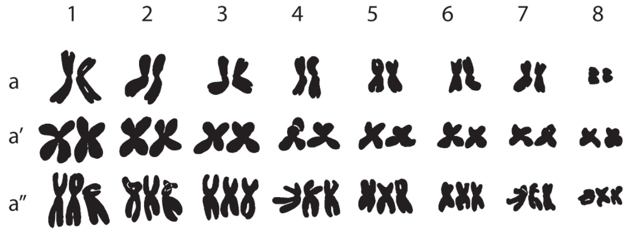

Fig. 6. – *Girardia tigrina*. Three new idiograms of population from Lake Biwa-ko (Station 11). a and a’: 2x = 16, with a karyotype of 2m + 2m + 2m + 2m + 2m + 2m + 2m + 2m. a’’: 3x=24, with a karyotype of 3m + 3m + 3m + 3m + 3m + 3m + 3m + 3m. Diploid and triploid karyotypes were found in different specimens.

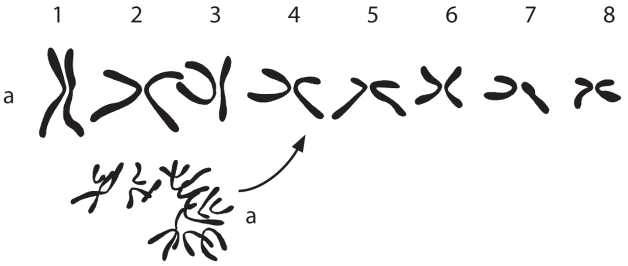

Fig. 7. – Metaphasic plates and idiograms of *Girardia tigrina* from Turin, Italy (idiograms reconstructed by Kawakatsu, based on data in Benazzi 1970).

Discussion. For the distribution of *G. tigrina*, Kenk (1974: 28) wrote: “North America, Mexico, Brazil, also introduced to Europe (widely distributed, including the British Isles), Israel, and Japan”. Later, *G. tigrina* was reported from several localities in Brazil, Uruguay, and additional localities in Japan (Kawakatsu et al. 1981a, b, 1982, 1983, 1985, 1986a, 2007b, c, 2008; Chinone et al., 2008). For additional data, including locality information, see Hirao et al. (1970), Kawakatsu et al. (2007c) (station 2); Kawakatsu et al. (1985) (stations 4 and 5); Tamura et al. (1985) (station 6); Kawakatsu et al. (1993b) (station 9); Nishino et al. (2002) (station 11); Kawakatsu et al. (2007b, c) (staion 15); Chinone et al. (2008) (station 16); Tanaka (2008) (station 18).

Girardia dorotocephala (Woodworth, 1897)

Material examined and distribution: Honshû: Stations 17, 19 (3 localities), and 20 (3 localities) (Fig. 1). Naturalized populations occur at stations 19 and 20.

Morphology. Living sexually mature specimens from the Hino (Station 19) and Sagamihara (Station 20) populations measure 12 -18 mm x 1.5 -2.5 mm. The large head is of a broad, triangular form with a pair of long and pointed tentacles. Two eyes, each surrounded by conspicuous, non-pigmented ocular areas, are located slightly anterior to the level of the base of the auricles (Kawakatsu et al. 2007b, 2008, 2009).

Ground colour of the dorsal surface uniform brown, with the central region of the body being uniformly grey, interspersed with many small, clear spots (Fig. 8).

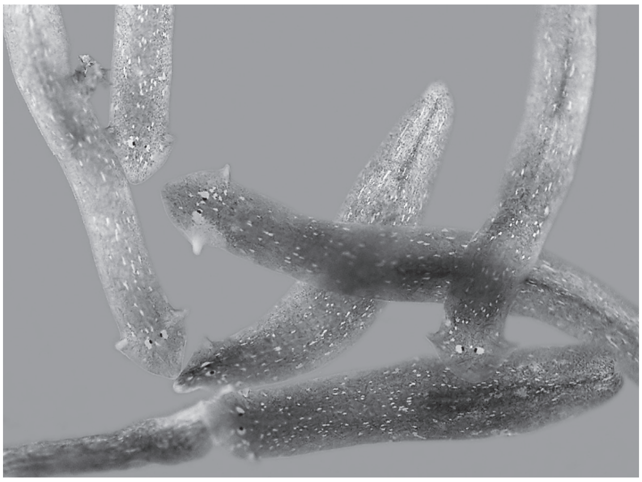

Fig. 8. – External features of *Girardia dorotocephala.*

The pharynx shows a brownish pigmentation; its outer musculature is provided with a third, extra layer of longitudinal muscle fibres, a feature that is characteristic for this species (Kawakatsu & Mitchell 1981). Testes ventral, throughout the body. For copulatory apparatus, see Fig. 9. Specimens from Japan have a short, conical and symmetrical penis papilla and a large, spherical penis bulb with spacious bulbar cavities.

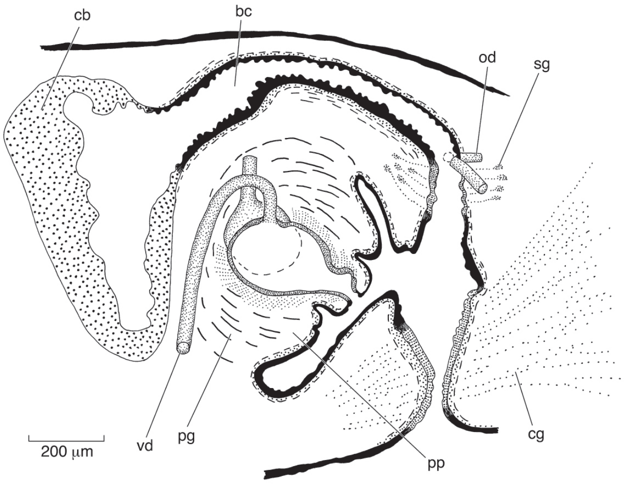

Fig. 9. – *Girardia dorotocephala.* ZMA V.Pl. 6813.1 (Station 20). Sagittal reconstruction of the copulatory apparatus. Abbreviations: bc, bursal canal; cb, copulatory bursa; cg, cement glands; od, oviduct; pg, penis glands; pp, penis papilla; sg, shell glands; vd, vas deferens.

Karyology. Three sets of idiograms of *G. dorotocephala* from three localities in Japan are given in Fig. 10. The karyotype of *G. dorotocephala* is 2x=16, with 8 pairs of metacentric chromosomes (Kawakatsu et al. 2007b, 2008). The diploid karyotype of *G. dorotocephala* resembles that of *G. tigrina*.

G. dorotocephala specimens from South Virginia, U.S.A. showed a diploid karyotype of 6 pairs of metacentric chromosomes and 2 pairs of submetacentric elements (Fig. 11) (Benazzi 1970).

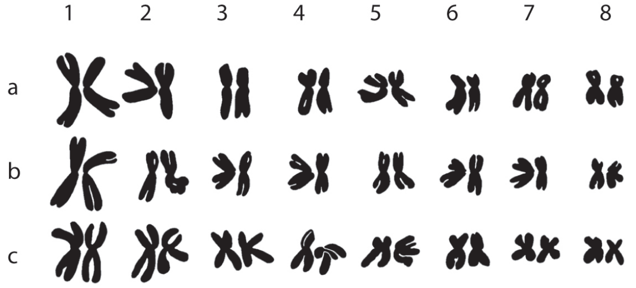

Fig. 10. – *Girardia dorotocephala.* Three idiograms for populations from Stations 17 (a), 19 (b), and 20 (c). a: 2x=16, with a karyotype of 2m + 2m + 2m + 2m + 2m + 2sm + 2sm + 2m. b and c: 2x=16, with a karyotype of 2m + 2m + 2m + 2m + 2m + 2m + 2m + 2m.

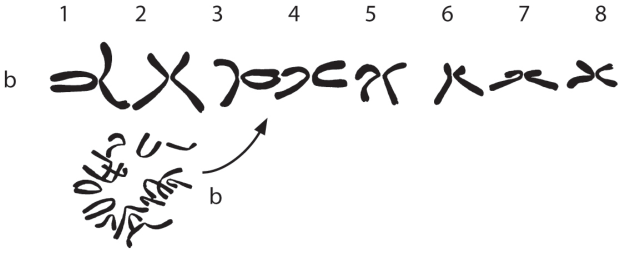

Fig. 11. – Metaphasic plate of *Girardia dorotocephala* from South Virginia, U.S.A. (idiograms reconstructed by Kawakatsu, based on data in BENAZZI 1970).

Discussion. The histological and morphological characteristics of the Japanese animals are in conformity with those of the North American animals (cf. Kawakatsu & Mitchell 1981). The native distribution of *G. dorotocephala* concerns North America, southern Canada, and Mexico (Kenk 1974). The species has been introduced into Hawaii (Kawakatsu et al. 1984). The occurrence of this North American species in Japan was noted promptly by Kawakatsu et al. (2007b, 2008). The present paper supports these earlier and preliminary identifications by providing a reconstruction of the copulatory apparatus of the Japanese representatives of *G. dorotocephala*. For additional data, including locality information, see Kawakatsu et al. 2007b, c, 2008) (station 17).

Rhodax evelinae? Marcus, 1946

Material examined and distribution: *R. evelinae*? has been reported from two stations (Fig. 1). Honshû: Nagoya City (Station 5). Hokkaidô; Sapporo City (Station 10) (cf. Kawakatsu et al. 1985, 1995).

Morphology. Living, asexual specimens of the Nagoya (Station 5) and Sapporo (Station 10) populations measure 3-5 mm x 0.4-0.5 mm. Head rounded, with a gentle swelling on either side. Eyes situated close together, each surrounded by a non-pigmented ocular area. Ground colour of the dorsal surface pale brown. Numerous, small, darkish pigments present at the pharyngeal region. Ventral surface pale. A slightly thi-ckened adhesive region present at the anteroventral end of the body (Fig. 12).

Fig. 12. – External features of *Rhodax evelinae?* a: dorsal view of entire animal; b; dorsal view of the anterior region.

Karyology. Two idiograms of *Rhodax evelinae*? from the Sapporo locality are shown in Fig. 13. The karyotype of asexual specimens of *Rhodax evelinae*? is 3x=24, with four pairs of metacentric chromosomes (1st, 2nd, 3rd, 6th) and four pairs of submetacentric chromosomes (4th, 5th, 7th, 8th pairs). A few B-chromosomes are also present. Idiograms **a** and **a'** concern two different somatic cells of a single specimen. see

Fig. 13. – *Rhodax evelinae*? Two idiograms of a population from Station 10 (a and a'), as reported by Kawakatsu et al. 1995); a and a' were found in somatic cells of a single specimen. a: 3x=24, with a karyotype of 3m + 3m + 3m + 3sm + 3sm + 3m + 3sm + 3sm. a': 3x + 1LB + 1SB =25 + 1SB, with a karyotype of 3m + 3m + 3m + 3sm + 3sm + 3m + 3sm + 3sm + 1LB + 1SB.

For idiograms of *R. evelinae*? from the Nagoya population, Kawakatsu et al. (1985) and Oki et al. (1995).

Discussion. *Rhodax evelinae*? was found only in aquaria for tropical fish culture (water temperature ≥ 20° C). In Brazil *Rhodax evelinae* Marcus, 1946 inhabits dirty ponds, rivers and clear-water brooks near the city of São Paulo (Marcus 1946). Recently, immature or asexual *Rhodax* sp. specimens have been found also throughout Rio Grande do Sul, southern Brazil, mainly in drainage ditches of rice fields but also in other types of wetlands. (A. M. Leal-Zanchet, pers. com.).

For additional data, including locality information, see Kawakatsu et al. (1985) (station 5) and Kawakatsu et al. (1995) (station 10).

GENERAL DISCUSSION

The following factors may have facilitated the introduction and subsequent geographical spread of exotic freshwater triclads in Japan:

(1) Popularization of domestic tropical fish cultures

After the 1960's various kinds of tropical fishes and aquatic plants have been imported increasingly in Japan from regions such as Hong Kong, Singapore, Australia, Europe (Germany and The Netherlands), South and East Africa, North America, South America (especially Peru and Brazil; cf. Kawakatsu et al. 1995). Various exotic species of shrimps, crabs, crayfishes, newts, frogs, and tortoises are now common in tropical fish stores and pet shops in Japan. Those freshwater pet organisms were frequently discharged into Japanese waters, where they now run out of control (artificial removal of exotic harmful organisms is prohibited by the Invasive Alien Species Act) (cf. Kawakatsu et al. 2007b, c, 2008).

The naturalized population of *D. austroasiatica* is recorded only from Kyôto (Mizoro-ga-ike Pond) in Central Japan. Since this presumably Southeast Asian species is an inhabitant of warm waters, an increase of its naturalized populations in Japan is expected to be minimal. In contrast, the naturalized populations of the two North American *Girardia* species*, G. tigrina* and *G. dorotocephala*, may increase rapidly in water systems of lowland areas in Southern and Central Japan. Under natural conditions in Southern and Central Japan these animals may propagate asexually by fission (*G. tigrina*) or sexually by cocoon-laying (*G. dorotocephala*). The collector of *G. dorotocephala* at the Hino locality observed many cocoons of this species in the field (cf. Kawakatsu et al. 2008).

(2) Culture of exotic aquatic animals as food

Case 1. In the middle of 1984 many specimens of *Dugesia austroasiatica* were collected from culture ponds of Tilapia fishes (*Oreochromis niloticus* (Linnaeus, 1758) from Africa) in Saga City, Kyûshû (St. 7) (cf. Kawakatsu et al. 1986b). Water temperature was ≥15°C, even in mid-winter. Many specimens of exotic planarians were attached to roots of the water hyacinth [*Eichhornia crassipes* (Martius) Solm, 1883] grown in these culture ponds.

Case 2. Cherax tenuimanus (Smith, 1912), an edible West Australian crayfish, was cultured at the Ibusuki Branch of the Kagoshima Prefectural Fisheries Experimental Station, Ibusuki City, Kyûshû. The culture ponds were fed by underground water, with temperatures ≥15°C, even in mid-winter. In the spring of 1985 many specimens of *G. tigrina* were collected from the body surface of a host crayfish (Station 6; cf. Tamura et al. 1985; Oki et al. 1995). Many specimens of *Temnosewellia minor* (Haswell, 1887) were also found on the body surface and gill chamber of these crayfishes(cf. Tamura et al. 1985; Kawakatsu et al. 2007a).

(3) Importation of live specimens of Chinese freshwater shrimps

Niwa & Ohtaka (2006) reported the occurrence of many specimens of the Chinese symbiotic branchiobdellidan, *Holtodrilus truncatus* (Liang, 1963) on shrimps from the Sugo River (Yumesaki River system), Honshû, Central Japan since 2003. The authors considered these shrimps (probably *Caridina denticulata* (De Haan, 1849) or a closely related species) to have been introduced from China for use as live baits in recreational fishing. A temnocephlid species, *Scutariella japonica* (Matjašič, 1990), has been recorded from Japanese shrimps [*Caridina denticulata* (De Haan, 1849)] at the same locality of *H. truncatus* in the Sugo River. At present, exotic planarians have not been recorded from the Sugo River locality. However, this kind of commercial activity may potentially accelerate the arrival of exotic freshwater planarians in Japan.

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Ultrastructure of germaria and vitellaria in *Dugesia sicula* Lepori, 1948 (Platyhelminthes, Tricladida, Paludicola)

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ABSTRACT. The female gonad of the diploid and sexual planarian *Dugesia sicula* Lepori, 1948 has been studied by means of transmission electron microscopy (TEM). The germaria consist of two ovaries occurring in the anterior body part behind the eyes. The vitellaria are composed of two lateral rows of vitelline follicles ranged dorsally and ventrally from the ovaries to the copulatory apparatus. During their growth, oocytes are provided with scattered residual yolk globules. The cytoplasm of maturing oocytes is filled with mitochondria, chromatoid bodies, Golgi complexes, RER, annulate lamellae and small yolk globules (2-3 µm in diameter) surrounded by a simple membrane, and lacks cortical granules. During the early stage of differentiation, vitellocytes enclose well-developed RER and Golgi complexes. Mature vitellocytes show yolk and eggshell globules, lipid droplets and glycogen particles. Golgi complexes and RER are involved in the production of eggshell and yolk globules respectively. Eggshell globules exhibit a concentric pattern typical for triclads. Results are discussed and compared with bibliographic data for other triclads.

KEY WORDS: Platyhelminthes, Paludicola, *Dugesia sicula*, oogenesis, vitellogenesis, ultrastructure.

INTRODUCTION

Previous ultrastructural studies of ovogenesis and vitellogenesis in Platyhelminthes have offered good insights into triclad phylogeny and classification. Among many works, the study of Gremigni (1979) strongly supported the classification proposed by Ball (1974) and confirmed that the primary taxonomic division in the Paludicola is between two groups; the first group comprises the Dugesiidae whose oocytes produce yolk globules of similar structure ranging from 2 to 7 µm in size with, for some species, a paracrystalline organization of the granular component (Gremigni, 1969a, 1974). The second group comprises the Planariidae and the Dendrocoelidae, whose oocytes produce a monolayer of cortical granules of almost 1µm diameter while yolk globules are absent (Gremigni & Domenici, 1975; Gremigni, 1974, 1979).

Dugesia sicula Lepori, 1948 is an important paludicolan species because it is represented by many asexual triploid populations distributed in the Mediterranean region that arose from diploid strains. These diploid strains are in danger of extinction especially in European countries and until now have not been the subject of fine ultrastructural studies. Gremigni (1979) presented only the structure of yolk globules occurring inside oocytes of an Italian strain of this species. So the aim of this study is to analyse, using ultrastructural techniques, the cytodifferentiation of oocytes and vitelline cells in a Tunisian diploid strain.

MATERIALS AND METHODS

Specimens of *Dugesia sicula* were collected from Mountain streams in North West Tunisia. It is a diploid sexual strain showing a high percentage of sexually-mature specimens and many fertile cocoons during the period of late spring to autumn. Worms were fixed in 3% glutaraldehyde in 0.2 M sodium-cacodylate buffer (pH 7.4) for about 4h at 4 ℃, washed in buffer for 30 min at 4 ℃, post fixed in 4% OsO_4 in cacodylate buffer for 1h at room temperature. They were dehydrated in an ethanol series, embedded in EPOX resin and polymerized at 60 ℃ for 12h. Ultrathin sections of the ovaries and vitellaria were stained with uranyl acetate and lead citrate and examined under a JEOL GEM-1010 at 80 kV.

RESULTS

Germarium morphology

Paired ovaries of *Dugesia sicula* are oval and located anteriorly to the pharynx in a ventral position behind the eyes. A distinct extracellular lamina is not observed, although a multilayered gonadal wall made by the extension of peripheral accessory cells separates each ovary from the surrounding parenchyma (Fig. 1). Accessory cells, usually associated with oocytes, are located at the periphery of the gonad and between germ cells. Peripheral accessory cells are elongated and their cytoplasm contains many mitochondria, a flattened nucleus and dense nucleoplasm with small patches of heterochromatin mainly adjacent to the inner nuclear membrane (Fig. 1). Internal accessory cells located between germ cells present the same structure as the peripheral ones but they are larger and their nuclear membrane is irregular (Fig. 1).

Oocyte differentiation

Oogonia and young oocytes are elliptical cells with a diameter of 15 to 20 µm. The large nucleus is surrounded by a thin dense ooplasm. It contains a prominent nucleolus, the chromatin is diffuse with few clumps scattered in the nucleoplasm (Fig. 2). The nucleolus is about 2 to 2.5 µm; it contains granules and intermingled fibrils (Fig. 2). In the perinuclear ooplasm, dense and granular material (0.2 to 0.6µm) usually called 'chromatoid bodies' is located facing the nuclear envelope pores, which are increased in number (Fig. 3). Few elongated mitochondria, free ribosomes and glycogen particles are present in the ooplasm. Golgi complex and endoplasmic reticulum (RER) are rare in these maturing oocytes.

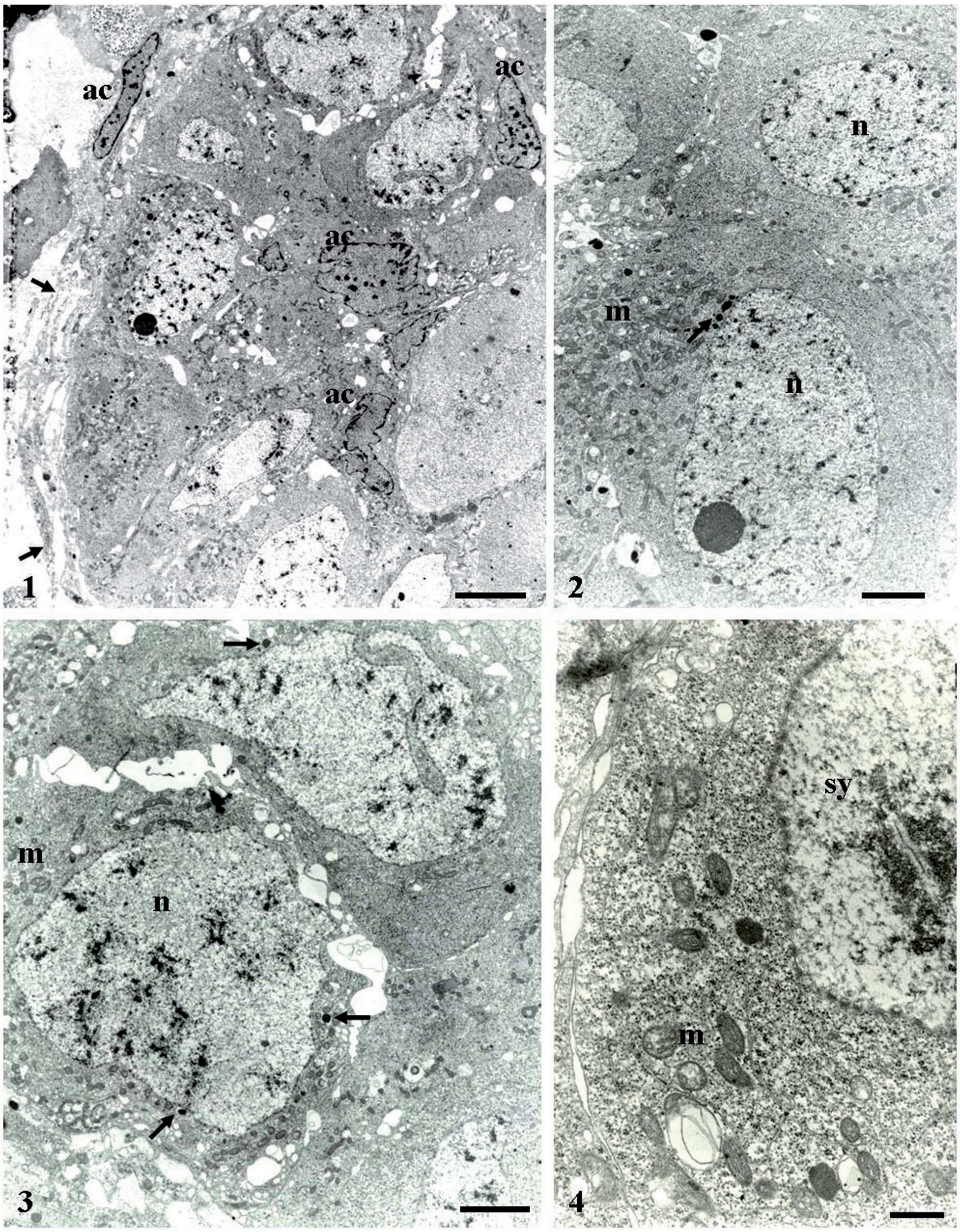

Fig. 1-4. – Ovogenesis of *Dugesia sicula*. Electron micrographs of sections through the ovary. **1. -** Thin tunic separates each ovary from the surrounding parenchyma (arrows). Elongated accessory cells (ac) associated with oocytes at the periphery of the gonad and between germ cells. Scale bar: 5µm. **2. -** Oogonia and young oocytes. The large nucleus (n) is surrounded by a thin basophilic cytoplasm rich in elongated mitochondria (m), free ribosomes, glycogen particles and chromatoid bodies (arrow). The nucleolus is prominent, the chromatin is diffuse. Scale bar: 2µm. **3. -** Maturing oocytes showing a small enlargement of cells, mitochondria (m) increase in number, chromatoid bodies are located facing the increasing nuclear envelope pores (arrow). Scale bar: 2µm. **4. -** Nucleoplasm of growing oocytes displaying synaptonemal complexes (sy) with the tripartite structure. Scale bar: 500nm.

In growing oocytes, nucleoplasm displays synaptonemal complexes with the tripartite structure typical of pachytene and diplotene stages (Fig. 4). As with Dugesiidae, endogenous vitellogenesis characterizes the oocyte differentiation. In fact this differentiation is marked by a slight enlargement of cells, a limited development of the RER and an increase of the Golgi complexes. The latter apparatus produces small vesicles, which undergo repeated and progressive coalescence giving rise to small round or oval yolk globules 2-3 µm in diameter surrounded by a simple membrane (Fig. 5). Mature yolk globules have a roundish, granular, electron-dense core surrounded by a thin layer of amorphous, translucent material (Fig. 6). Two points are worthy of note; firstly, the granular component occupies practically all the globule whereas the amorphous material is confined to the peripheral area. Secondly, no paracrystalline organization was observed in *D. sicula* oocyte yolk globules.

Yolk globules are scattered throughout mature oocyte cytoplasm, and they are surrounded by a great number of mitochondria clusters close to chromatoid bodies. This mitochondria proliferation constitutes a large 'Balbiani body' or 'yolk nucleus' (Fig. 8) which may include lipid droplets of 3.5 µm diameter and few bundles of annulate lamellae (Fig. 7).

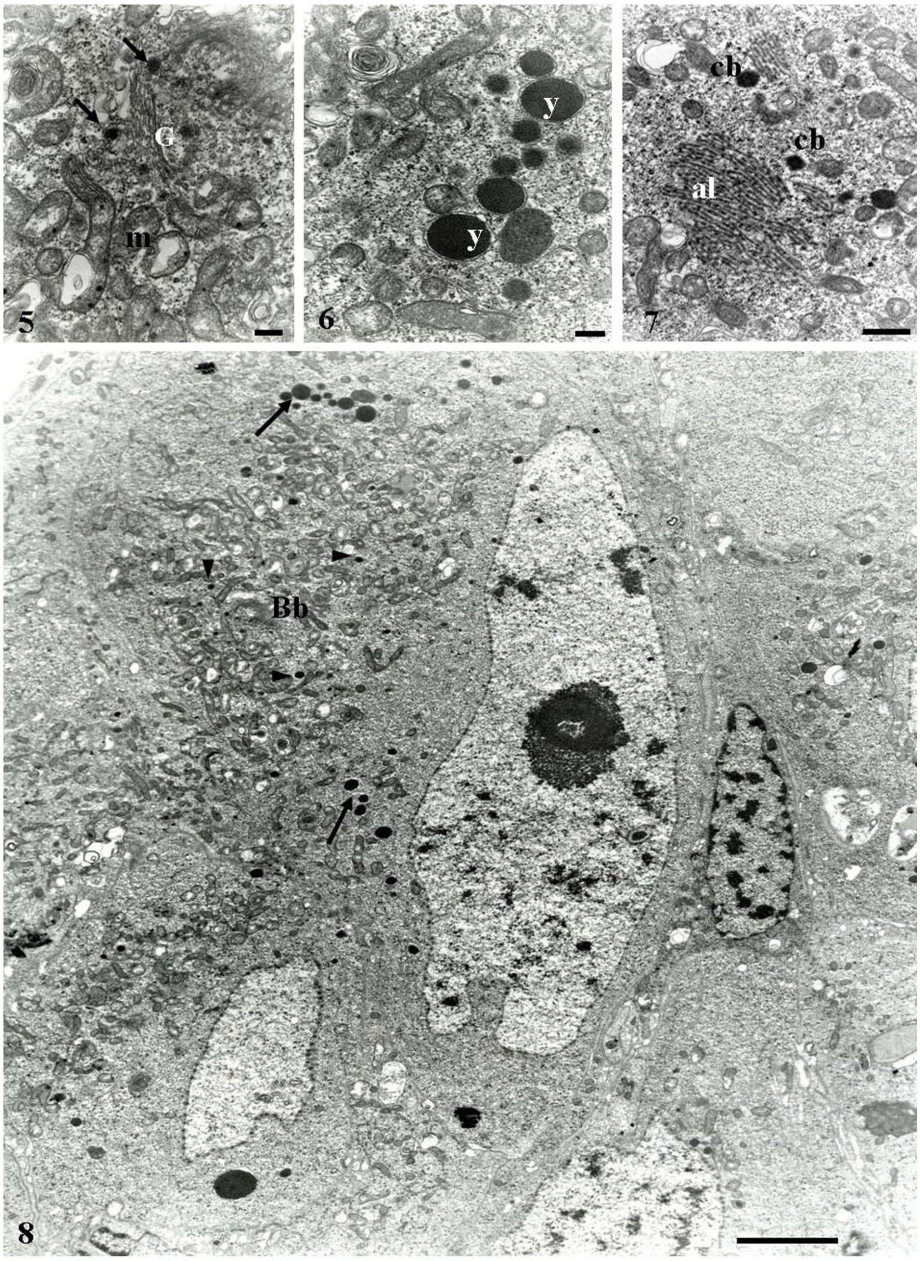

Fig. 5-8. – Ovogenesis of *Dugesia sicula*. Electron micrographs of sections through maturing oocytes. **5.** - Golgi complex (G) produces small vesicles that fuse to give rise to small yolk globules (arrows). Mitochondrion (m). Scale bar: 200nm. **6.** - Small round or oval yolk globules (y) 2-3µm diameter surrounded by a simple membrane accumulate in the ooplasm. Scale bar: 200nm. **7.** - High magnification within Balbiani body showing few bundles of annulate lamellae (al), mitochondria and chromatoid bodies (cb). Scale bar: 500nm. **8.** - Balbiani body (Bb) in maturing oocytes. Yolk globules (arrows) are scattered throughout ooplasm and surrounded by a great number of mitochondrial clusters close to chromatoid bodies (arrow heads). Scale bar: 2µm.

Vitellocyte structure

In vitelline follicles, young vitelline cells are peripheral and display the same shape as undifferentiated cells (neoblasts) scattered everywhere in the surrounding parenchyma. They are oval shaped, 10-12 µm long and have a large rounded nucleus (5-6 µm diameter) surrounded by a thin basophilic cytoplasm. In the nucleoplasm, the chromatin is diffused and the nucleolus displays fibrillar and granular components. The cytoplasm contains many free ribosomes, some mitochondria and few RER.

During their differentiation, vitelline cells are characterized by a very low nucleoplasmic ratio and have the typical structure of secreting cells: They show a notable increase in RER, Golgi complex and lipid droplets (Figs 9, 10). Differentiated vitelline cells are spherical or cylindrical and can reach 30-40 µm in diameter. Their cytoplasm contains low numbers of free ribosomes with mitochondria and very developed cisterns of RER (Figs 9, 11). The nucleus reduces

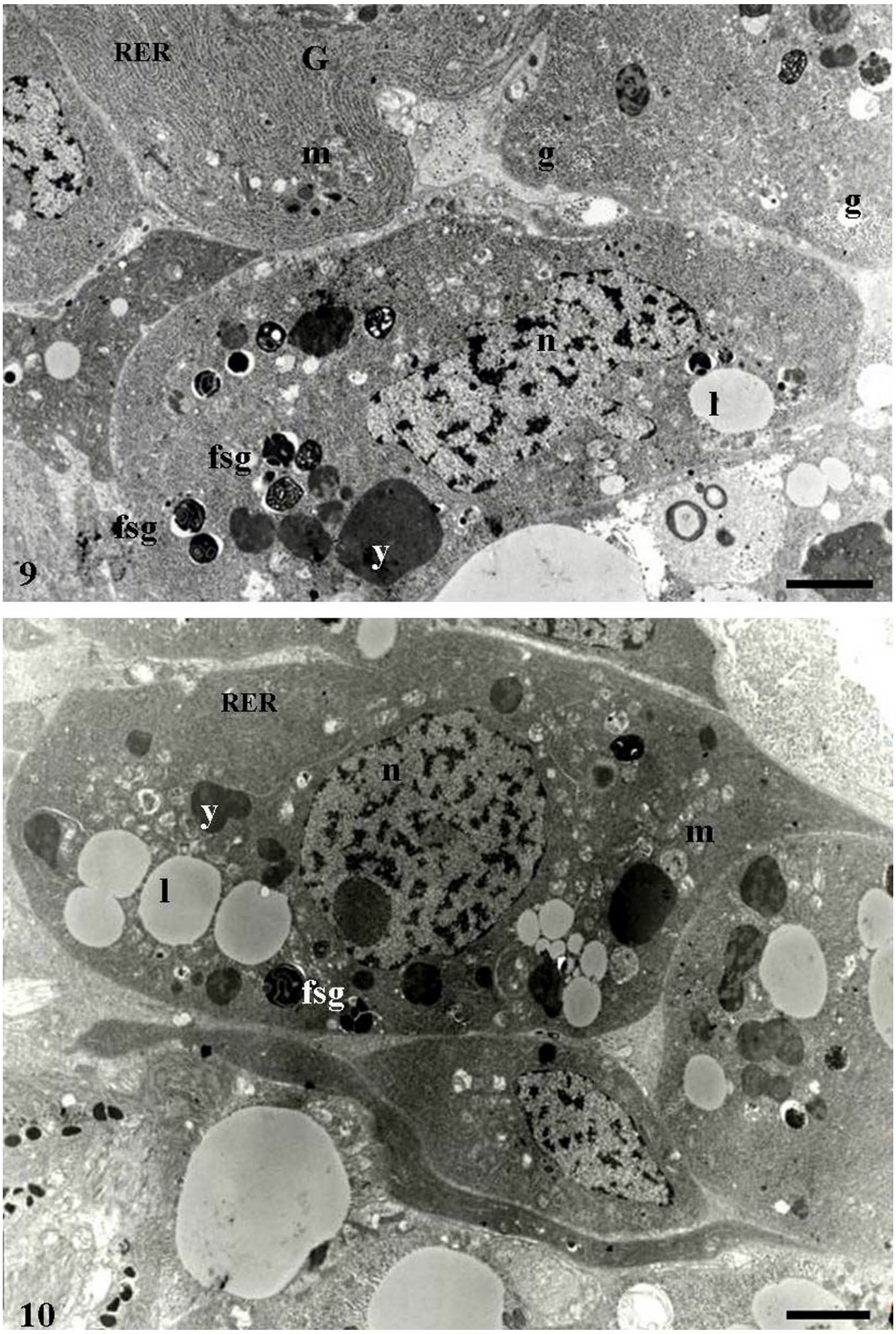

Fig. 9-10. – Vitellogenesis of *Dugesia sicula*. Electron micrographs of sections through maturing vitellocytes. The cytoplasm contains low numbers of free ribosomes, mitochondria (m), very developed cisterns of the RER, glycogen particles (g), lipid droplets (l), yolk (y) and forming eggshell globules (fsg). Nucleus (n). Scale bars: 5µm and 2µm.

in size, the chromatin is diffused and a small nucleolus displaying a granular component is present (Fig. 10). The cytoplasm of mature vitelline cells is almost entirely occupied by yolk and shell globules, lipid droplets and glycogen granules. Lipid droplets of 1-1.5 µm are spherical, larger and less numerous than yolk and shell globules (Figs 12, 13).

Yolk globules result from the coalescence and repeated fusion of vesicles of 0.2 µm diameter produced by the RER and containing only one type of homogeneous component of medium density. Their final diameter varies roughly between 1.5 and 2.5 µm (Fig. 12). Eggshell globules are less numerous than yolk globules and derive from fusion of Golgi-derived vesicles of medium electron density (Fig. 11). At the beginning of eggshell globule formation, these two types of vesicles agglomerate to give globules containing small dense bodies and broad masses of fairly dense homogeneous material (Figs 12, 13). Immature eggshell globules, limited by a simple membrane, show a multigranular pattern (Figs 12, 13). In mature eggshell globules, of 0.8-2 µm diameter, two components of different density are present giving it a typical organization of alternating dense and clear concentric rings (Fig. 14).

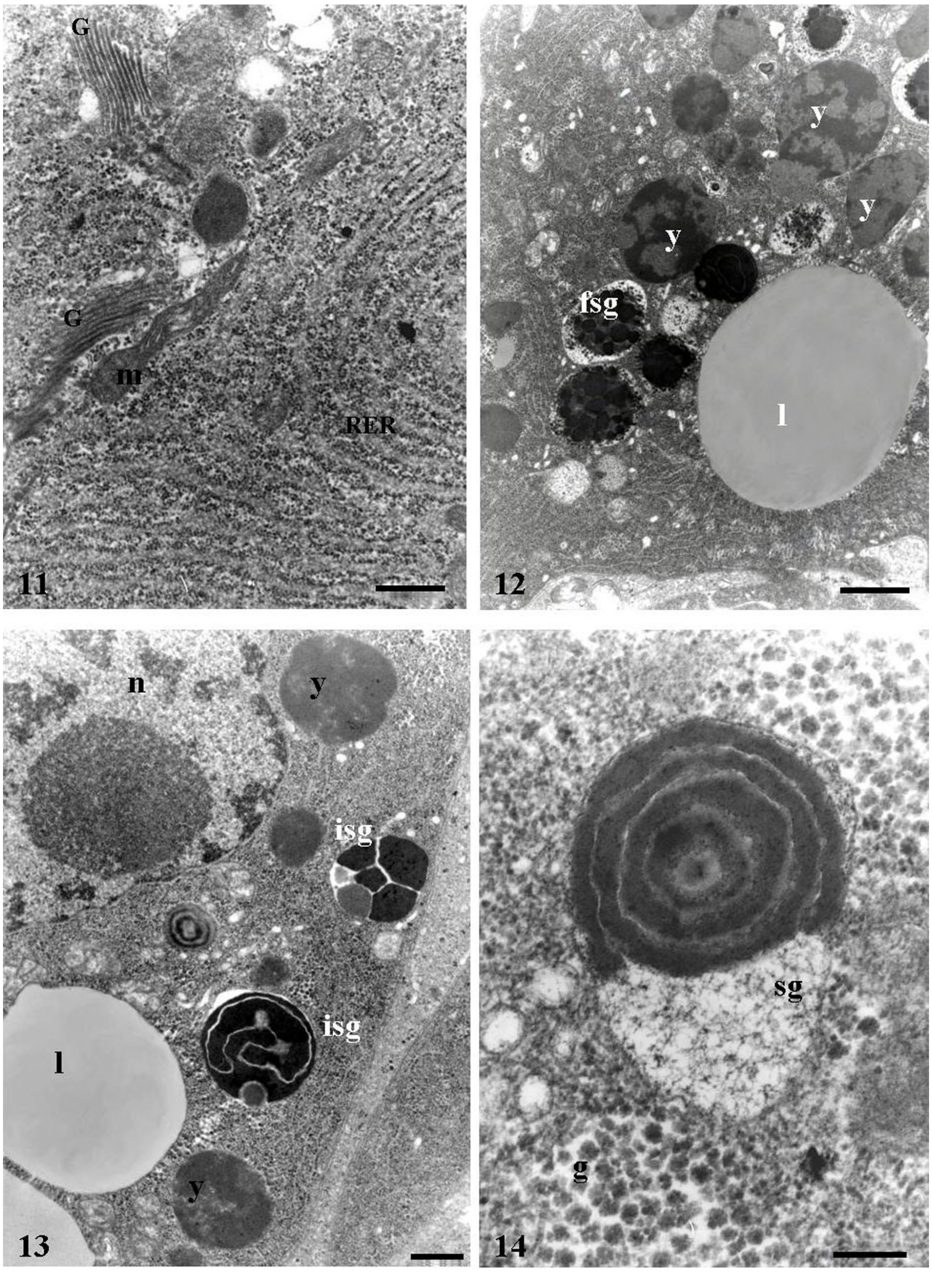

Fig. 11-14 – High magnification of cytoplasm of mature vitelline cells. **11.** - Note the development of Golgi complexes (G) producing small granules of medium electron density. Long cisterns of endoplasmic reticulum (RER) occupy a great part of the cytoplasm. Mitochondrion (m). Scale bar: 500nm. **12.** - Cytoplasm of mature vitelline cells showing yolk globules (y), lipid droplets (l) and forming shell globules (fsg). Scale bar: 1µm. **13.** - Cytoplasm of mature vitelline cells showing yolk globules (y) and immature eggshell globules (isg) limited by a simple membrane and displaying a multigranular pattern. n, nucleus. Scale bar: 500nm. **14.** - Maturing eggshell globule (sg) showing the typical organization of alternating dense and clear concentric rings. g, glycogen. Scale bar: 200nm.

DISCUSSION

The female gonad of *D. sicula* consists of well-separated germarian and vitellarian areas both enveloped only by extension of accessory cells; an extracellular lamina is lacking. The heterocellular female gonad of turbellarians can reportedly be enveloped by a tunica composed of an outer extracellular lamina and an inner sheath of accessory cells. This tunica has been found in some Proseriata (Sopott-Ehlers, 1986, 1994, 1995), Rhabdocoela (Lucchesi et al., 1995; Sopott-Ehlers, 1997; Falleni et al. 1998, 2002, 2005) and recently in three terricola; *Geoplana burmeisteri* Schultze & Müller, 1857 *Microplana scharffi* Graff, 1896 and *Microplana terrestris* Müller, 1774 (Falleni et al., 2006, 2009). This extracellular lamina is absent in the marine triclad *Sabussovia dioica* Claparède, 1863 (Tekaya et al., 1999) and in Lecithoepitheliata (Falleni et al., 1995; Falleni, 1997).

Accessory cells have been observed to surround oocytes and vitellocytes during their differentiation and they are thought to have a protective function and to play a trophic role in transferring precursors from the surrounding tissue to the maturing oocytes (Nigro & Gremigni, 1987; Falleni & Gremigni, 1992; Falleni et al., 2002, 2006, 2009). They have been reported throughout oogenesis in some Proseriata (Sopott-Ehelers, 1994, 1995), Tricladida (Gremigni & Nigro, 1983; Tekaya et al., 1999; Falleni et al., 2006, 2009) and Rhabdocoela (Falleni et al., 2002).

The synaptonemal complexes present in the nuclei of large oocytes indicate that their maturation is completed during the prophase of the first meiotic division. This phenomenon is marked also by the development of RER and Golgi complexes, which is correlated with the production of granules with a finely granular content of medium electron density. Such granules having a glycoprotein content remain scattered in the ooplasm throughout oogenesis and are interpreted as yolk; they have been observed in some proseriates (Falleni & Gremigni, 1992), freshwater triclads (Gremigni, 1969a, 1988) and terrestrial triclads (Falleni et al., 2006, 2009). This yolk production occurs only by an autosynthetic mechanism, as previously described in platyhelminths belonging either to the archoophoran or to the neoophoran level of organization of the female gonad (Gremigni & Falleni, 1992).

With respect to the shape of yolk globules produced within oocytes, Gremigni (1979) has noted that they are large in *D. sicula* and can reach a final diameter of 7-8 µm and more. However, in this work we have never observed such size. In fact, yolk globules produced in Tunisian *D. sicula* oocytes did not exceed 3µm as it was shown in *Dugesia dorotocephala* Woodworth, 1897 *Dugesia anceps* Kenk, 1930 and *Dugesia tigrina* Girard, 1850 studied by Gremigni (1979). As in *Dugesia benazzii* Lepori, 1951, *Dugesia biblica* Benazzi & Banchetti, 1973 and *Dugesia gonocephala* Dugès, 1830 (Gremigni, 1979), these yolk globules produced by *D. sicula* (this work) are scant and do not display the paracrystalline organization in the completely mature globules. The amorphous component appears to be rarefied, in the same way in both growing and mature globules, which thus allows the central granular component to appear surrounded by a thin translucent zone.

It can be noted that the structure of yolk globules produced in oocytes is quite different from those produced by vitelline cells. The oocyte yolk autosynthesis is considered as a character more primitive than the vitellaria yolk production and it is interpreted as a residue of an ancestral feature (Boyer, 1972).

Our ultrastructural study shows that a Balbiani body is present in growing oocytes of *D. sicula*. It consists of an accumulation in the ooplasm of many mitochondria, small electron-dense bodies and few annulate lamellae. This structure attracted the attention of many scientists who discussed its role and its composition in each zoological group. Previously it was believed that this cellular structure, which generally disappears after fecundation, intervenes in the yolk synthesis, but electron microscopy did not confirm this interpretation. Gremigni (1976) has indicated the presence of the Balbiani body in vitellogenic oocytes of *D. dorotocephala*. He noticed that this structure, with its typical organization, remains present in the prophasic oocytes ready to enter the oviducts. However, according to the same author, the Balbiani body cannot be regarded either as a constant structure with a well-defined function in the oocytes (since it is absent in *Dugesia lugubris* Schmidt, 1861, *D. gonocephala* and *D. benazzii* which produce yolk globules) or as possessing a direct role in yolk synthesis (since it is absent in vitelline cells of the neoophorans).

The presence of chromatoid bodies is a common aspect in differentiating cells, especially in the germline cells of many animals, as is the case in the oocytes of many Platyhelminthes (Gremigni, 1976; Justine & Mattei, 1986; Falleni & Gremigni, 1992; Falleni & Lucchesi, 1992; Tekaya, 1999; Falleni et al., 2002, 2006, 2009). Chromatoid bodies are maintained in germ cells during their differentiation from neoblasts and they are suggested to be concerned with the totipotency of theses cells (Shibata et al., 1999). It has been demonstrated that they contain many components as the transcript of genes implicated in germ cell development (Sato et al. 2006).

No types of cortical or peripheral granules have been found in the mature oocytes of *D. sicula* as in other Dugesiidae studied (Gremigni, 1969a, 1976, 1979, 1988). Such granules have been detected in the cortical ooplasm of marine (Gremigni & Nigro, 1983; Tekaya et al., 1999) and freshwater planarians belonging to the Planariidae and Dendrocoelidae (Gremigni, 1969b, 1979; Gremigni & Domenici, 1975) and in some proseriates (Gremigni & Nigro, 1984; Gremigni et al., 1986; Sopott-Ehlers, 1995).

Generally, the pattern of vitellocyte maturation in *D. sicula* is similar to that described in other neoophoran Platyhelminthes (Rieger et al., 1991) and especially in some marine, terrestrial and freshwater triclads (Domenici & Gremigni, 1974; Tekaya et al., 1998; Falleni et al., 2006, 2009). In fact the ultrastructural study of the vitellaria indicates that vitellocytes have the general structure of neoophoran vitelline cells. Young cells have a large nucleus with a prominent nucleolus and little-differentiated cytoplasm. During maturation, the nucleoplasmic ratio decreases gradually while the Golgi complex and the RER develop. The mature vitellocytes have a differentiated cytoplasm due to various inclusions: lipid droplets, yolk and eggshell globules. The mature eggshell globules display a substructrure similar to that observed in some proseriates, freshwater, terrestrial and marine triclads (Gremigni, 1988; Tekaya et al., 1998; Falleni et al., 2006, 2009) where two components display concentric rings of respectively dense and clear materials. According to previous studies on freshwater planarians of the Dugesiidae family, the clear component of these eggshell globules corresponds to non-phenolic proteins while the dense component contains polyphenols (Gremigni & Domenici, 1974).

Three types of eggshell globules have been described in neoophoran Platyhelminthes: the homogeneous pattern typical of Lecithoepitheliata and some Proseriata (Gremigni, 1988; Falleni et al., 1995), the convoluted or concentric pattern typical of some Proseriata and Tricladida (Gremigni & Domenici, 1974; Gremigni & Falleni, 1991; Sopott-Ehlers, 1991, 1995; Tekaya et al., 1998; Falleni et al., 2006, 2009) and the multigranular pattern typical of the Prolecithophora and Rhabdocoela (Gremigni, 1988; Gremigni & Falleni, 1991, 1998; Sopott-Ehlers, 1997; Falleni et al., 2005).

It is known that vitelline cells produce proteins and polyphenols needed for the formation of the sclerotin eggshell through a tanning process (Marinelli, 1972; Gremigni & Domenici, 1974; Yanagita & Yamamoto, 1981). Mature vitellocytes contain other types of reserve material such as lipids, glycogen particles and yolk globules. These latter constitute the second type of membrane-bound inclusion and are produced by the RER and Golgi complex. They have a homogeneous content of medium electron density and they are similar to those described in other triclads (Gremigni & Falleni, 1992; Falleni et al., 2005, 2006, 2009).

In conclusion, the present ultrastructural investigation has provided evidence that the female gonad of *D. sicula* displays ultrastructural features typical of the basic pattern of Tricladida and especially of the freshwater planarians from the Dugesiidae family. Since many triploid asexual populations of this species are present in the Mediterranean region and can develop ex-fissiparous and sterile specimens, we envisage a similar study of their female gonad to compare it with that of the sexual strain.

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Spermatogenesis and spermatozoon ultrastructure in *Dugesia sicula* Lepori, 1948 (Platyhelminthes, Tricladida, Paludicola)

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ABSTRACT. We examine for the first time spermatogenesis, spermiogenesis and spermatozoon ultrastructure in *Dugesia sicula* Lepori, 1948 a sexual and diploid planarian living in Tunisian springs. This TEM-study shows that early spermatids joined by cytophores have rounded nuclei. During spermiogenesis, a row of microtubules appears in the differentiation zone beneath the plasma membrane and close to the intercentriolar body, which consists of several dense bands connected by filaments. Two free flagella (9+1 configuration) grow outside the spermatid. An apical layer of dense nucleoplasm develops and the flagellum appear, facing in opposite directions before rotating to lie parallel to each other after the intercentriolar body splits into two halves. Mitochondria are closely packed around the spermatocyte nucleus before fusing during spermiogenesis, to form a long mitochondrion, which lies parallel to the elongated nucleus along the ripe spermatozoon. The latter is thread-shaped and consists of two regions: the proximal process and a distal part. The former contains the nucleus and a part of the mitochondrion. The latter contains the rest of the mitochondrion and a tapering tail of the nucleus. Separation between these two regions is marked externally by the insertion zone of the two free flagella. The flagella extend posteriorly along the distal part of the spermatozoon. The spermatozoon nucleus consists of a lucent and a dense component coiled in a screw-like pattern around each other. The single row of peripheral microtubules consists of a maximum 40 microtubules in the middle part with an internal layer of three supplementary microtubules.

KEY WORDS: Platyhelminthes, Tricladida, Paludicola, *Dugesia sicula*, testis, ultrastructure.

INTRODUCTION

Electronic microscopy is very useful in spermatozoon and spermatogenesis studies aiming to elucidate phylogenetic relationships among Platyhelminthes (Tyler et al., 1986; Raikova, 1991; Rieger et al. 1991; Watson & Rhode, 1995, Watson, 1999, 2001). Many papers have provided knowledge already acquired on this subject with revisions on general assumptions about Platyhelminthes phylogeny (Hendelberg, 1969, 1983, 1986; Ehlers, 1985; Rohde, 1990; Watson & Rhode, 1995, Watson, 1999, 2001).

With respect to triclads, investigations have been carried out on the spermatogenesis of freshwater planarians. Klima (1961) published electronic micrographs of spermatogenesis and the spermatozoon ultrastructure in paludicolan triclads but without explaining the process of spermiogenesis. Parts of the process were studied in *Dugesia tigrina* Girard, 1850 (Silveira & Porter, 1964), *Dendrocoelum lacteum* Müller, 1774 and *Planaria torva* Müller, 1774 (Hendelberg, 1969), *Polycelis tenuis* Ijima, 1884 and *Polycelis nigra* Müller, 1774 (Franquinet & Lender, 1972, 1973), *Dugesia lugubris* Schmidt, 1861 (Farnesi et al., 1977) and other species (Ishida & Teshirogi, 1988; Ishida et al., 1991). Li et al. (1992) described for the first time the spermatozoon and the spermiogenesis ultrastructure in the terricolan triclad *Artioposthia sp.* Rhode & Watson (1995) studied the ultrastructure of sperm and spermiogenesis of the paludicolan *Romankenkius libidinosus* Sluys & Rohde, 1991 and an unidentified maricolan. The spermatogenesis of the gonochoric maricolan triclad *Sabussowia dioica* Claparède, 1863 has been studied using light and electron microscopy by Tekaya & Zghal (2001).

The Platyhelminthes spermatozoa are generally elongated and thread-like. They differ enormously from the presumed primitive and modified forms encountered in other animal groups. They lack a distinct head, an intermediate part and a tail. Flagella can be free or incorporated in the spermatozoon body, or are even lacking (as in Macrostomida and Prolecithophora). The present study reports the first ultrastructure data regarding spermatogenesis, spermiogenesis and spermatozoon structure in *Dugesia sicula* Lepori, 1948 a diploid paludicolan strain from Tunisian springs.

MATERIALS AND METHODS

Specimens of *D. sicula* were collected from a spring located in the Serj Mountain northwest of Tunisia. There is a large sexual population of sexually mature, young and newly hatching individuals raised from cocoons deposited under stones. For transmission electron microscopy (TEM), parts of specimens were fixed in 3% glutaraldehyde in 0.2 M sodium-cacodylate buffer (pH 7.4) for about 4h at 4°C, washed in buffer for 30 min at 4 °C, post fixed in 4% OsO_4 in cacodylate buffer for 1h at room temperature. After dehydration through graded ethanol and propylene oxide, pieces were embedded in Epox. Ultrathin sections through testes, sperm ducts and seminal vesicle were stained with uranyl acetate and lead citrate and examined under a JEOL GEM-1010 at 80 kV.

RESULTS

Spermatogenesis

The testicular follicles of *D. sicula* are situated dorsally throughout the body. They are rounded or oval shaped and can reach 45µm diameter. At least one layer of parietal cells, as they have been called by Hendelberg (1983), delimits the testis follicles sharply from the surrounding parenchyma. These parietal cells display lobed nuclei, prominent nucleoli, numerous mitochondria, well-developed endoplasmic reticulum (ER), lipid droplets and electron-dense granules. All stages of germ cells from spermatogonia to spermatozoa are present at the same time. They extend from the peripheral side to the lumen. Clusters of spermatogonia and spermatocytes are close to the parietal cells, whereas groups of spermatids and spermatozoa are free in the gonad lumen.

Spermatogonia have almost the same shape as neoblasts: little cytoplasm and large nuclei containing granular and fibrillar chromatin (Fig. 2). Spermatocytes are connected by cytoplasmic bridges. Within these germ cells, mitochondria are rich in cristae and increase in number (Fig. 3). Annulate lamellae of various sizes appear and prominent Golgi complexes become very close to the nuclei (Fig. 3). Spermatids are maintained together by cytoplasmic bridges consequent upon incomplete cell division during spermatogenesis. Anucleate pieces of residual cytoplasm following spermatid detachment are also present in the lumen (Fig. 1).

Spermiogenesis

Within early spermatids, the rounded nucleus occupies the distal end of the cell and becomes gradually condensed. Small dense granules appear inside the nucleus in close contact with chromatin (Figs 4, 5). Nuclear pores are prominent in the region closest to the differentiation zone (Fig. 5). The latter is a small cytoplasmic protrusion that develops distal to the nucleus and where an intercentriolar body (ICB) is built to support the two flagella growing out in opposite directions (Figs 6, 8). Spermatids change their spherical shape to become pear-shaped and the nuclei become increasingly elongated and filiform. During the first spermiogenesis stages, mitochondria encircle the nucleus, a row of microtubules appears under the plasma membrane in the differentiation zone, and a dense layer of nucleoplasm develops in the apical region of the nucleus (Figs 6-8). The ICB appears initially with an irregular outline; it contains dense granules and some translucent regions (Fig. 6). The final ICB consists of five bands connected by fine filaments; one dense and thick central band, two intermediate interrupted bands on both sides of it and finally and more externally two thin and continuous ones (Figs 7, 8). It appears that both flagella, lengthening in opposite directions outside the spermatid, are not directly fixed to the ICB. On the contrary, their basal bodies are separated by a small space from the external bands of it. However, these basal bodies are attached with the help of small dense plates to the plasma membrane, and with the help of rootlets to the nearest nuclear membrane (Fig. 8). During advanced stages of spermiogenesis, spermatids start to elongate and their shafts grow in the distal direction. The nucleus elongates too and cytoplasm containing mitochondria and other inclusions migrates alongside the nucleus. Thus, the cell attains gradually the filiform shape of the ripe spermatozoon. The ICB splits at its central band allowing both flagella to rotate in order to approach and to lie parallel to each other (Fig. 9). Each flagellum remains attached with its basal body to one of the basal parts of the intercentriolar body.

Spermatozoon structure

The ripe spermatozoon examined in the testis lumen and vasa deferentia is thread-like and shows two parts: a proximal main body containing the nucleus and the mitochondrion, and a distal process containing mainly the mitochondrion. The elongated nucleoplasm consists of two components; one dense and filamentous (chromatin) and another more lucent (probably protein) coiled around each other in a screw-like pattern (Fig. 10). Mitochondria fuse end-to-end to form a single elongated mitochondrion lying alongside the nucleus of the mature sperm. In some sections, the mitochondrion and the nucleus appear coiled around each other in a screw-like fashion (Fig. 10). Both free flagella are subterminal and emerge together from one side of the spermatozoon between the proximal and the distal parts (Figs 11, 12). We distinguished fusion between flagella only for a short distance just after the insertion zone (Fig. 12). The axoneme pattern is of 9+1; many dense granules (probably glycogen) are present between the nine external microtubule doublets and the central complex (Fig. 13). Flagellar tips split into long microvilli containing few microtubules (Fig. 13).

A single row of peripheral longitudinal microtubules with a maximum number of 40 surrounds the nucleus and the mitochondrion along the entire sperm shaft (Figs 14-17). A short row of three inner microtubules extends beside the mitochondrion and the nucleus along the sperm shaft before disappearing toward both ends (Figs 14, 15). Within vasa deferentia, cross sections through ripe spermatozoa permitted distinction between proximal and distal parts. The nucleus tapers in sections through the distal part where we can see mainly the mitochondrion surrounded by peripheral microtubules (Fig. 16). Very close to the proximal end, the mitochondrion becomes very small in cross sections, and we can see only the nucleus surrounded by peripheral microtubules decreasing in number (Fig. 17).

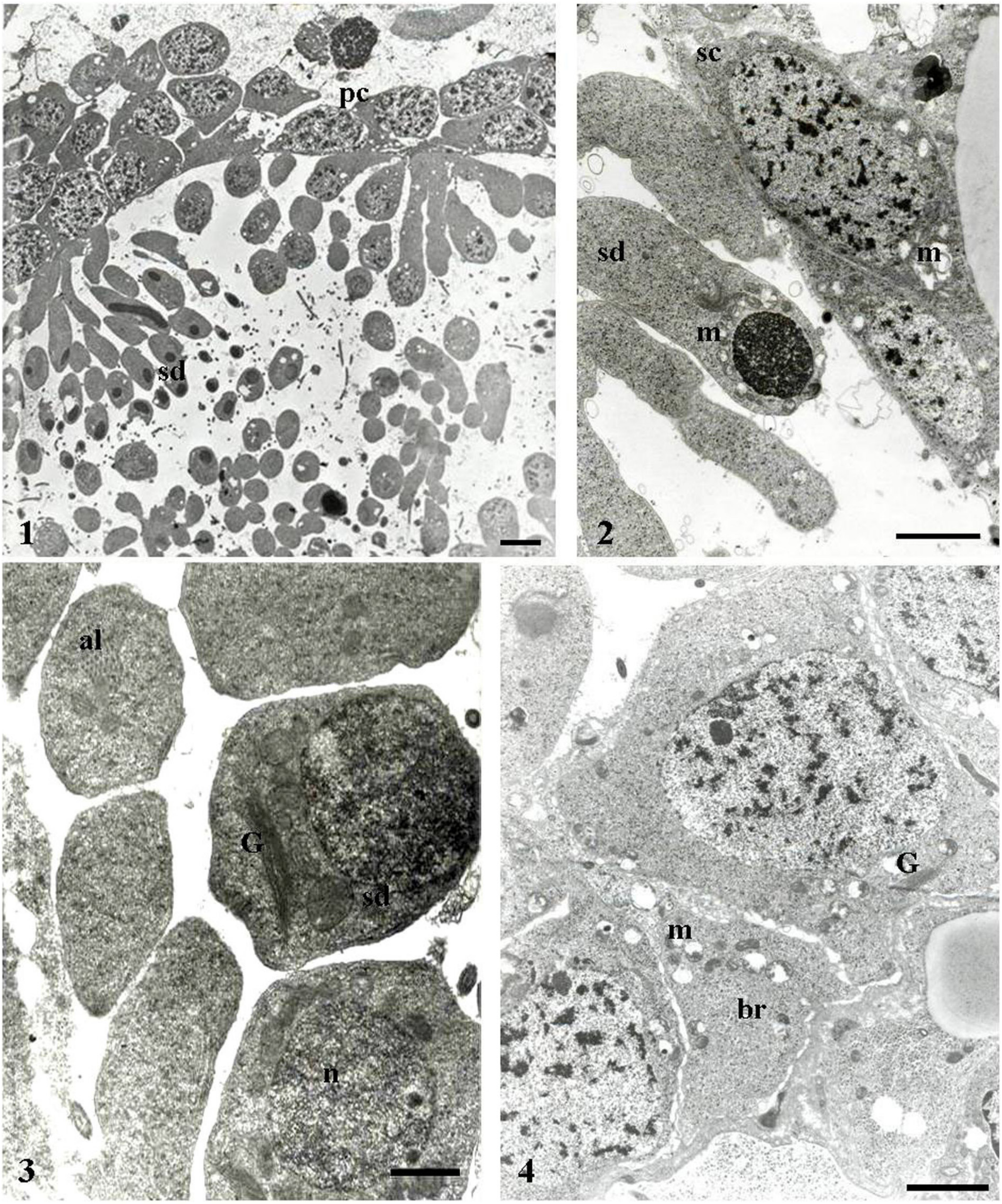
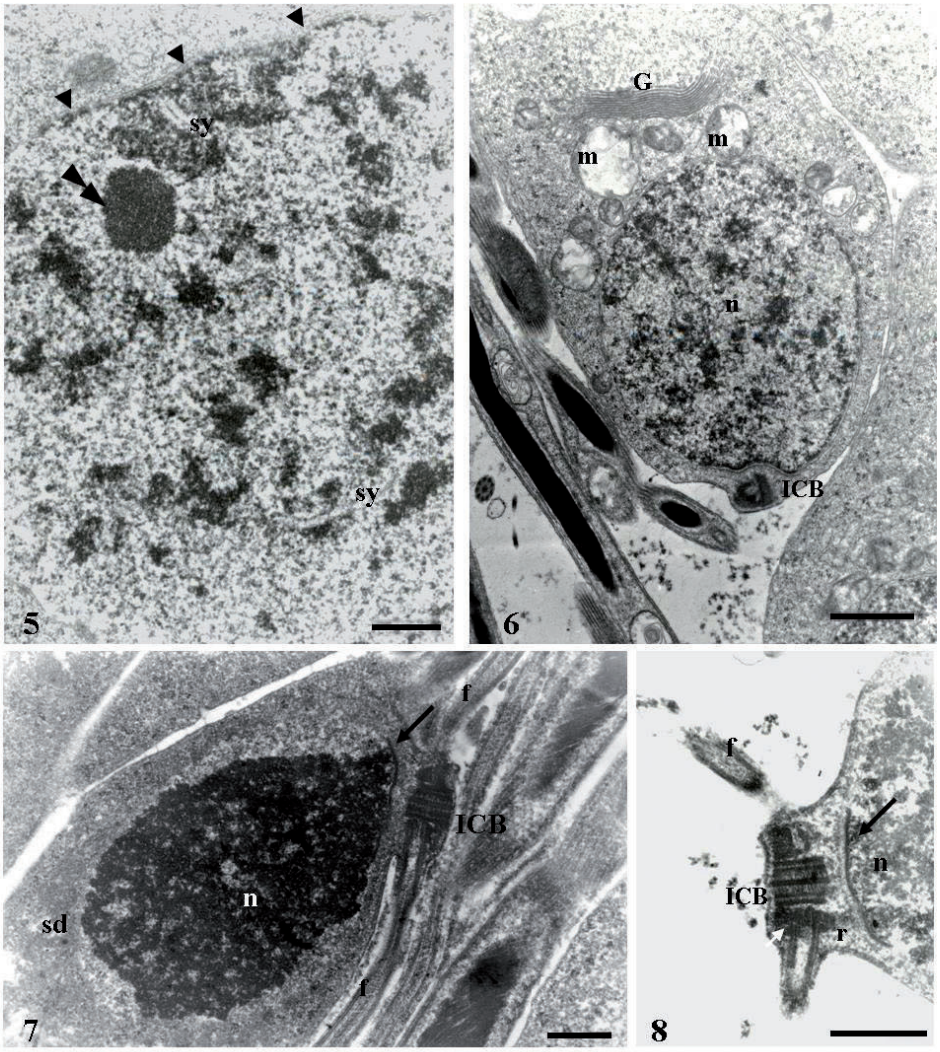


Fig. 1-4. – Spermatogenesis of Dugesia sicula. 1. - Electron micrograph of a section through a testis follicle showing parietal cells (pc) and different stages of male germ cells in the testis lumen. Scale bar = 5µm. 2. - Spermatocytes (sc) are connected to each other by cell processes and their cytoplasm is rich in mitochondria (m). Within young spermatids (sd), mitochondria are rich in cristae and increase in number. Scale bar = 2 µm. 3. - Annulate lamellae (al) of various sizes appear and prominent Golgi complexes (G) become very close to the nuclei (n). Scale bar = 1 µm. 4. - Spermatocytes are connected to each other by cytoplasmic bridges (br). Scale bar = 2 µm.



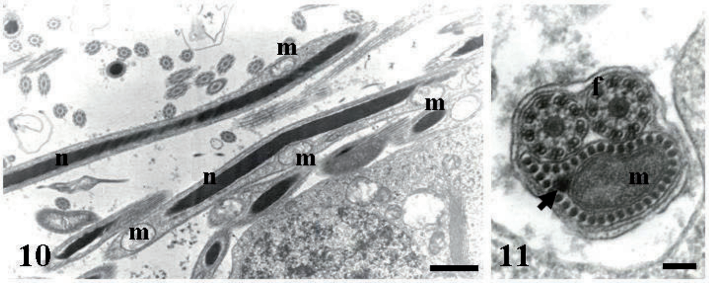


Fig. 5-9. – Spermiogenesis of *Dugesia sicula.* **5. -** Early spermatid, Small dense granules are in close contact with chromatin inside the nucleus (double arrow head), synaptonemal complexes (sy) appear and nuclear pores are prominent (arrow heads). Scale bar = 1 µm. **6. -** Early spermatid, the rounded nucleus occupies the distal end of the cell and becomes gradually condensed, a differentiation zone appears distal to the nucleus as a small protrusion of cytoplasm where the intercentriolar body (ICB) develops to support the two flagella. G; Golgi complexes, m: mitochondrion. Scale bar = 1 µm. **7-8. -** An apical layer of dense nucleoplasm develops (black arrows). The final ICB consists of five bands connected by fine filaments; one thick central and dense band, two intermediate interrupted bands on both sides of it and finally and more externally two thin and continuous ones. Both flagella (f) grow in opposite directions outside the spermatid (sd). Their basal bodies are attached with the help of small dense plates to the plasma membrane (white arrow), and with the help of rootlets (r) to the nearest nuclear membrane. n; nucleus. Scale bars = 500 nm. **9. -** The ICB splits at its central band allowing both flagella to rotate and to lie parallel to each other. Scale bar = 200 nm.

Fig. 10-11. – Spermatozoa of *Dugesia sicula* examined in the testes lumen. **10. -** The elongated nucleoplasm consists of one dense filamentous component and another more lucent coiled around each other in a screw-like pattern. The single elongated mitochondrion (m) lies alongside the nucleus; they appear coiled around each other in a screw-like fashion. n; nucleus. Scale bar = 1 µm**. 11. -** Transverse section in mature spermatozoa showing two free flagella (f), mitochondrion (m), a part of the nucleus (arrow) and microtubules. Scale bar = 100 nm.

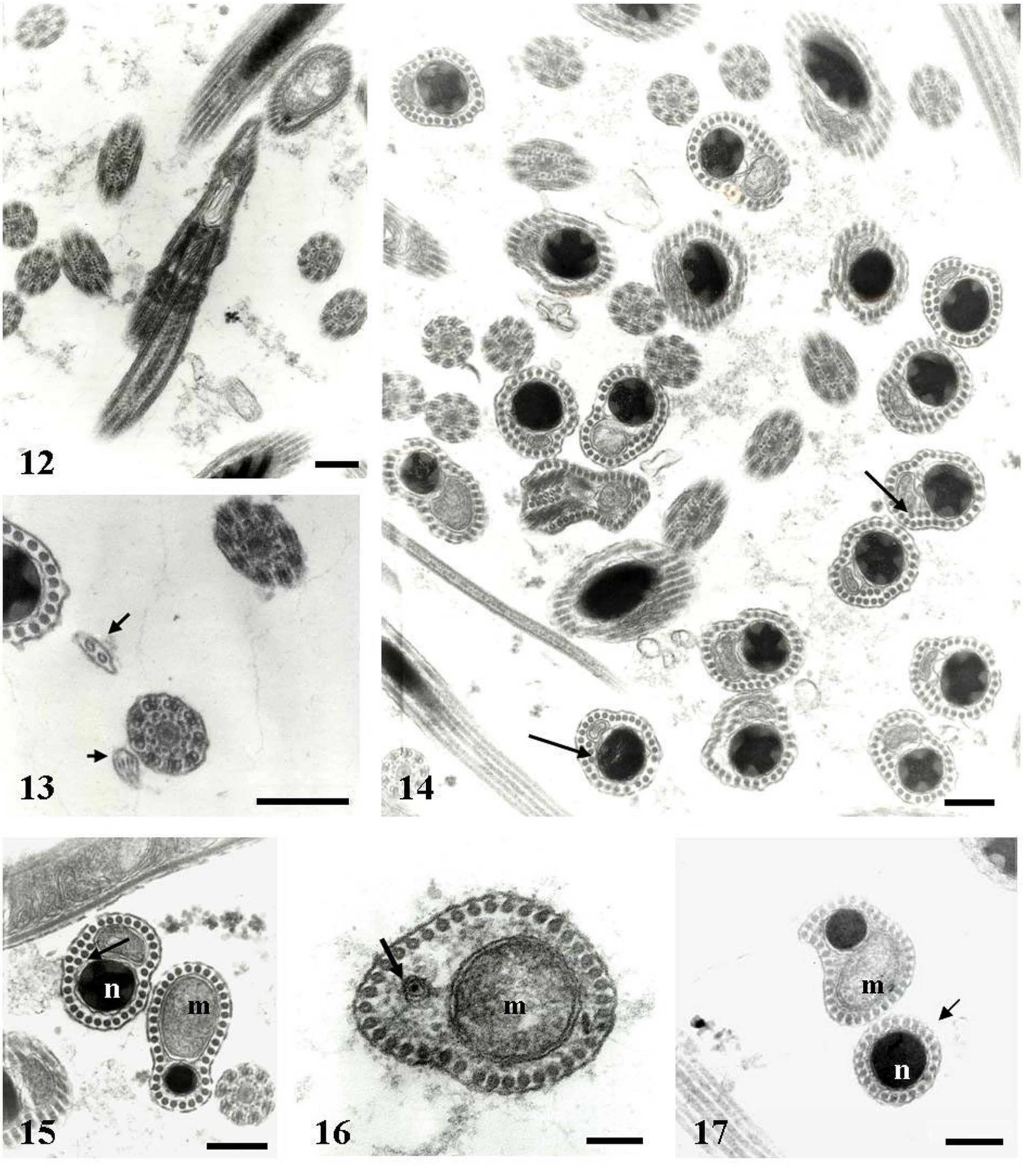


Fig. 12-17. – Spermatozoa of *Dugesia sicula.* Mature spermatozoa in the seminal vesicle. **12. -** Both flagella are free, subterminal and emerge together from one side of the spermatozoon; they fuse for a short distance just after the insertion zone before lying parallel to one another. Scale bar = 200 nm. **13. -** The axonemal pattern is of 9+1, many dense granules are present between the nine external microtubule doublets and the central complex. Tips of flagella split into long microvilli showing few microtubules in transverse sections (arrows). Scale bar = 100 nm. **14-15. -** Portion of the seminal vesicle showing different sections at different levels of spermatozoa. Flagella are free and we note the occurrence of three inner microtubules (arrows) close to the nucleus (n) and the mitochondrion (m). Scale bars = 200 nm. **16. -** Section through the distal part of the spermatozoon showing the mitochondrion (m) surrounded by peripheral microtubules and a tip of the elongated nucleus (arrow). Scale bar = 100 nm. **17. -** Cross section of the proximal end of the spermatozoon (arrow) showing only the nucleus (n) surrounded by peripheral microtubules. Scale bar = 100 nm.

DISCUSSION

In comparison with previous studies carried out on triclads, we enumerate the characteristics of spermatogenesis and spermiogenesis in the Tunisian sexual strain of *D. sicula*:

- Spermatids present an apical layer of dense nucleoplasm opposite to the differentiation zone. It has been interpreted as a basal plate by Ishida et al. (1991) and it has been found in paludicolan, maricolan and terricolan triclads (Franquinet & Lender, 1972; Ishida et al., 1991; Li et al., 1992; Rohde & Watson, 1995).
- Apart from in triclads, the presence of the rootlets has been mentioned for some Proseriates (Sopott-Ehlers, 1986, 1989, 1993) and many other taxa belonging to Trepaxonemata (see Watson & Rohde, 1995).
- During spermiogenesis, dense plates are associated with the ICB at the opposite side of the rootlets and around the basal bodies. These structures were observed in other triclads (Franquinet & Lender, 1972; Li et al., 1992; Rohde & Watson, 1995).
- The spermatozoon of *D. sicula* consists of two main parts: the proximal part, which contains the nucleus and the mitochondrion, and the distal process, which contains mainly the mitochondrion. Two free flagella emerge from the same side between the distal process and the proximal part. This spermatozoon form is known in several paludicolan and maricolan triclads (Silveira & Porter, 1964; Franquinet & Lender, 1972; Ehlers, 1985; Ishida & Teshirogi, 1988; Ishida et al., 1991; Rohde & Watson, 1995; Tekaya & Zghal, 2001).
- The organisation of the dense and lucent components of the nucleoplasm in *D. sicula* sperm was observed in other triclads too (Silveira & Porter, 1964; Ishida & Teshirogi, 1988; Ishida et al., 1991; Li et al., 1992; Rohde & Watson, 1995; Tekaya & Zghal, 2001).
- During spermiogenesis mitochondria fuse to form a single long mitochondrion, which lies parallel to the spermatozoon nucleus in a screw-like fashion in the proximal part before extending into in the distal part. Such organisation characterizes many triclads (Franquinet & Lender, 1972; Ishida et al., 1991; Rohde & Watson, 1995; Tekaya & Zghal, 2001).
- The internal layer of three microtubules present in *D. sicula* has been described in some other paludicolan and maricolan triclads (Farnesi et al., 1977; Ishida et al., 1991; Rohde & Watson, 1995).
- Flagella of *D. sicula* contain dense granules as in other turbellarians (Silveira & Porter, 1964; Li et al., 1992; Rohde & Watson, 1995).
- In mature sperm, tips of the flagella split into microvilli containing few microtubules as described in other triclads (Franquinet & Lender, 1972; Farnesi et al., 1977; Ishida & Teshirogi, 1988; Ishida et al., 1991; Li et al., 1992; Rohde & Watson, 1995).
- We notice that *D. sicula* sperm, along with some other turbellarian species and all Neodermata, lack the dense bodies characterizing several turbellarians (Ehlers, 1985).

In conclusion, this ultrastructural study carried out for the first time on the spermatogenesis and spermiogenesis of *D. sicula* enriches the knowledge in this field and shows that they are in conformity with previous studies on Platyhelminthes and especially in triclads.

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Replication of basal bodies during ciliogenesis in the epidermis of Prolecithophora and Lecithoepitheliata (Plathelminthes)

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ABSTRACT. Centriologenesis has been little studied in the Plathelminthes. Taking into account the importance of ultrastructural features for phylogenetic reconstructions, we studied the development of cilia in mature turbellarians *Friedmaniella* sp. (Prolecithophora), *Geocentrophora wagini,* and *Geocentrophora intersticialis* (Lecithoepitheliata) to compare the events of centriolo- and ciliogenesis in their epidermis. In all these species the formation of the multitude of centrioles follows the acentriolar pathway. In *Friedmaniella* sp., each centriole appears inside an individual filamentous accumulation. In *G. wagini*, the procentrioles arise in clusters of fibrous granules. Several procentrioles are usually produced in a separate cluster. Such a very big cluster of fibrous granules was also found in one cyton of *G. intersticialis.* The assemblage of cilia starts on the basal bodies with rootlets either in the cytoplasm of the epidermal layer or on the cell surface, after these basal bodies dock with the apical cell membrane. The last developmental mode is the only one found in epidermis of *G. intersticialis*. Both our results and the literature data suggest that the knowledge of ciliogenesis could assist in reconstructing phylogenetic trees of Plathelminthes.

KEY WORDS: centriole, ciliogenesis, fibrous granules, flatworms

INTRODUCTION

Epidermal ciliation pattern is considered as a main autapomorphy of the phylum Plathelminthes (Ehlers, 1985, 1986; Baguñà & Riutort, 2004), however ciliogenesis has been little studied in the flatworms. The importance of ultrastructural features for internal phylogeny of the Plathelminthes is evident (Rieger, 1981). It was shown that distinctive features of cilia in metazoans have significance for systematics (Tyler, 1979). Could the morphological aspects of ciliogenesis in the Plathelminthes be considered as phylogenetically significant? Here I tried to answer this question. In this paper evidence of the ciliogenesis in Prolecithophora and Lecithoepitheliata is presented and the available data of ciliogenesis in Acoela, Catenulida, Macrostomida, and Rhabdocoela are mentioned (Tyler, 1981, 1984; Soltynska et al., 1976; Ehlers, 1992; Cifrian et al., 1992). Preliminary results of the study of centriologenesis in *Friedmaniella* sp. and *Geocentrophora wagini* have been published earlier (Drobysheva, 1996, 2006).

MATERIALS AND METHODS

Adult specimens of *Friedmaniella* sp., *Geocentrophora wagini* Timoshkin, 1984*,* and *Geocentrophora intersticialis* Timoshkin, 1984 were collected from Lake Baikal. For electron microscopy, samples were fixed in 2.5% glutaraldehyde, followed by 2% osmium tetroxide. All fixatives were buffered to pH 7.4 with 0.05 M cacodylate. Further preparation was by standard methods for transmission electron microscopy. Ultrathin sections were examined using a LEO 100 and a JEM 1200-EX electron microscopes.

RESULTS

The epidermis of *Friedmaniella* sp. was a monolayered, cellular, and ciliated epithelium with a well developed basement membrane, separating the epidermis from the inner tissues (Drobysheva & Mamkaev, 1995) (Fig.1 A, B). The epidermis of *G. wagini,* and *G. intersticialis* was composed of insunk cellular epithelium (Drobysheva & Timoshkin, 2006; Timoshkin, 1991). In these species each epidermal cell consisted of a thin epidermal plate located over the basement membrane, and the massive nucleated cell body (cyton) in the parenchyma (Figs 2, 3). The epidermal plate and the cyton were connected by a thin cytoplasmic bridge penetrating the basement membrane (Fig. 2 A). The whole body surface of *G. wagini* was covered with cilia, while only the ventral side of the body bore cilia in *G. intersticialis.* In all three species the microtubules of ciliary axonemes showed an ordinary 9 + 2 pattern and two striated rootlets (a long vertical and a short horizontal) extended from each basal body. The epidermal cells of the studied turbellarians often contained the various structures typical of differentiating ciliated cells, including free centrioles, rootlets, and axonemes.

Many single centrioles and small centriole groups were scattered throughout the epidermal cell cytoplasm of *Friedma-*

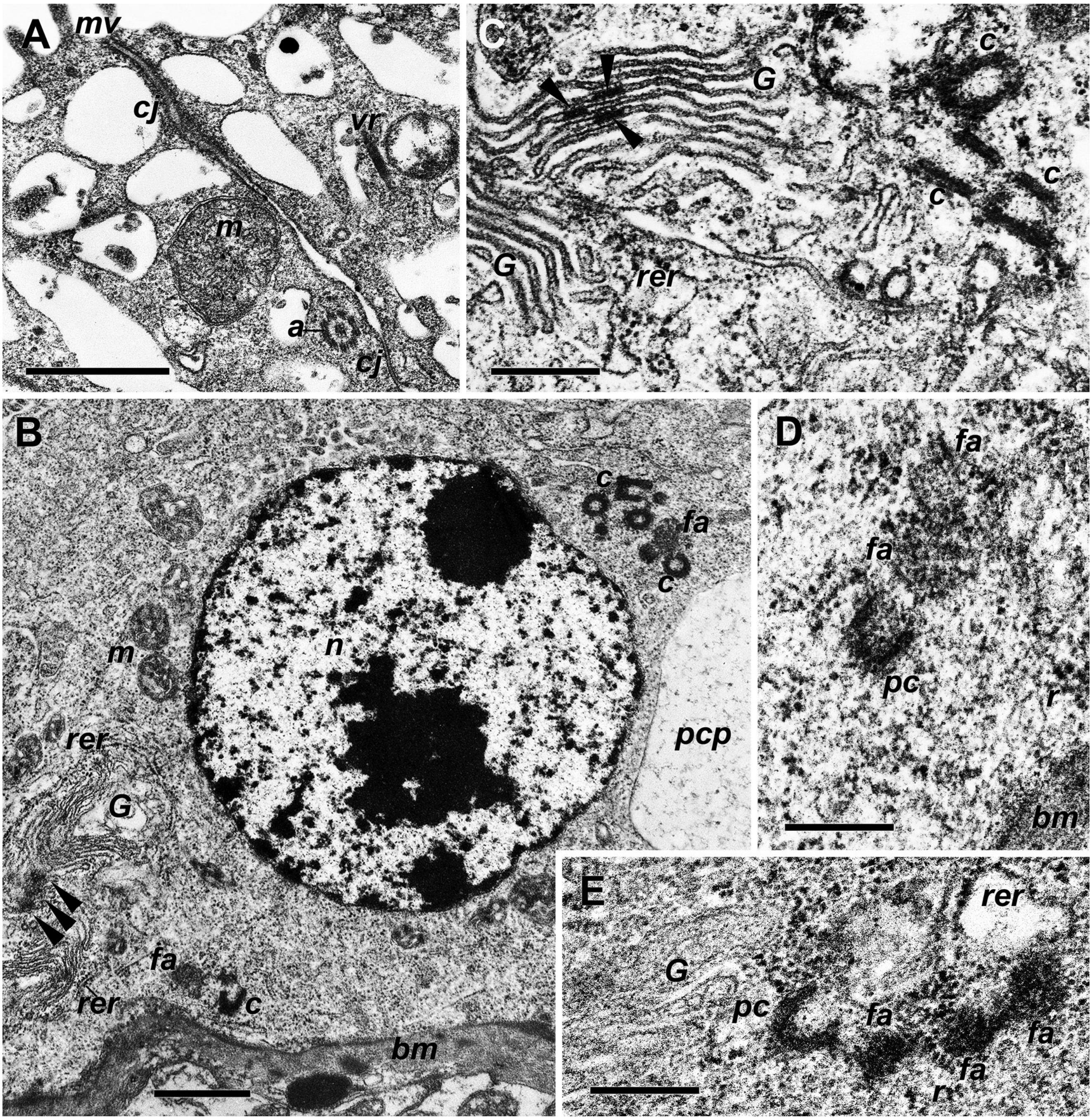

Fig. 1. – *Friedmaniella* sp. Fragments of epidermal cells. (A) Intracellular axoneme and cell junctions between two epidermal cells. (B) Basal part of epidermal cell with nucleus, Golgi apparatus, centrioles, and filamentous accumulations. (C) Golgi apparatus and centrioles. (D, E) Procentrioles and filamentous accumulations. a, axoneme; bm, basement membrane; c, centriole; cj, cell junction; fa, filamentous accumulations; G, Golgi apparatus; m, mitochondria; mv, microvilli; n, nucleus of epidermal cell; pc, procentriole; pcp, parenchymal cell process; r, free ribosomes; rer, profiles of rough endoplasmic reticulum; vr, vertical rootlet; arrowheads mark parts of Golgi apparatus cisternae with electron-dense contents (C). Scale bars = 1 µm (A, B), 0.5 µm (C–E).

niella sp., while intracellular axonemes and rootlets occurred more rarely (Drobysheva, 1996) (Fig. 1 A-E). Moreover, some relatively amorphous structures resembling local cytoplasmic condensations were found in the epidermal cells of *Friedmaniella* sp. (Fig. 1 A, D, E). Such structures appeared to consist of interlacing filaments. These filamentous accumulations were not membrane-enclosed and had a more -or -less spherical shape with diameter of about 200-300 nm. Centrioles and filamentous accumulations formed mixed groups (Fig. 1 D, E). Subsequent stages of centriole maturation suggested that each centriole arose inside an individual filamentous accumulation.

In the epidermal cells of *G. wagini*, centrioles were found

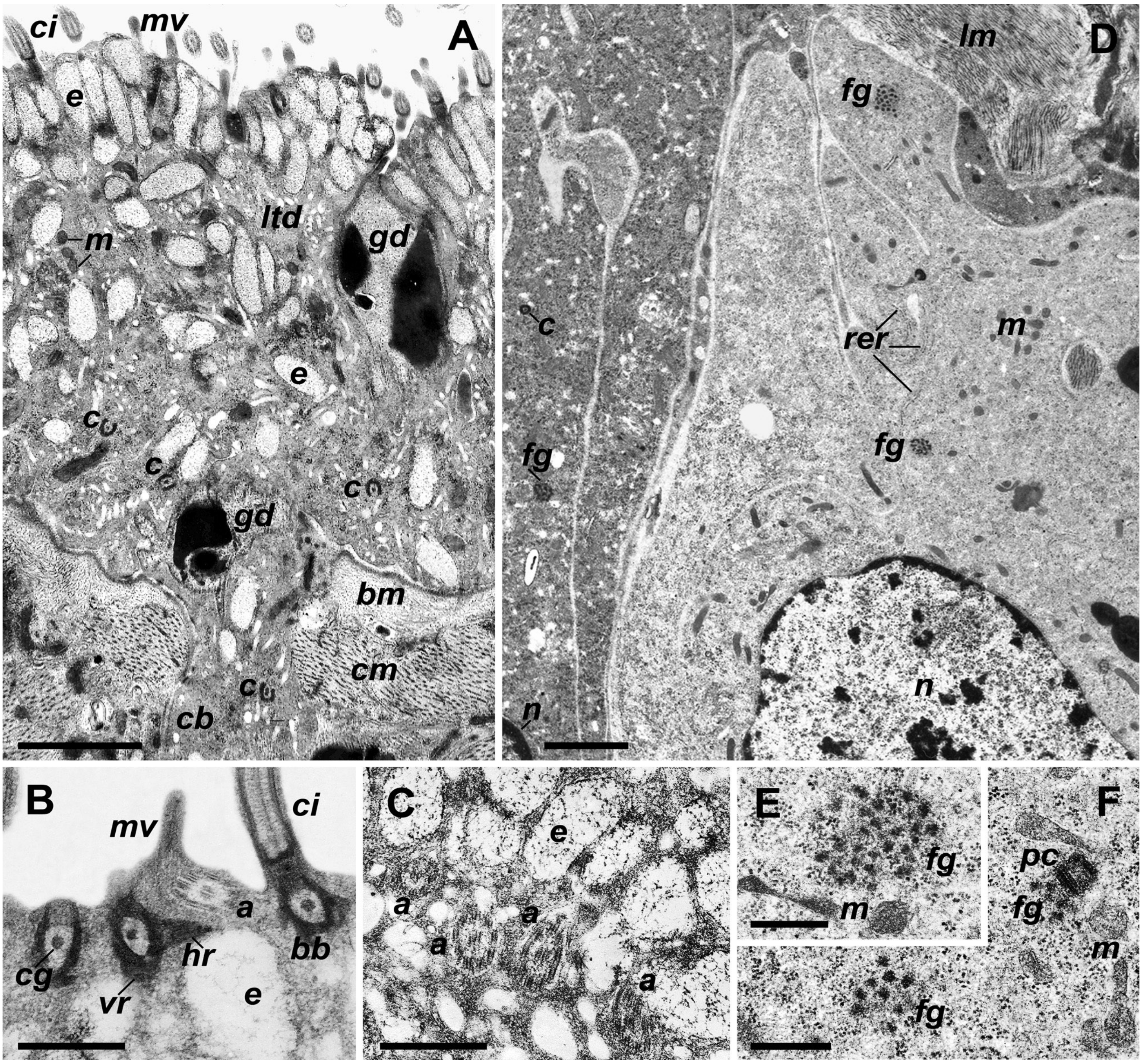

Fig. 2. – *Geocentrophora wagini*. Fragments of epithelial layer and cytons. (A) Epidermal plate connected to insunk part of epidermal cell (cyton) by a cytoplasmic bridge, as seen on the oblique section of ventral body side. (B, C) Apical zone of epidermal plates with cilium, basal bodies, and intracellular axonemes. (D) Parts of dark and light cytons with clusters of fibrous granules. (E, F) Clusters of fibrous granules and one procentriole at higher magnification. a, axoneme; bb, basal body; bm, basement membrane; c, centriole; cb, cytoplasmic bridge; cg, central granule in centriolar cylinder; ci, cilia; cm, circular muscles; e, epitheliosome; fg, fibrous granules; gd, parenchymal gland duct; hr, horizontal rootlet; lm, longitudinal muscles; ltd, light tubules and vesicles; m, mitochondria; mv, microvilli; n, nucleus of epidermal cell; pc, procentriole; rer, rough endoplasmic reticulum; vr, vertical rootlet. Scale bars = 2 µm (A, D), 0.5 µm (B, C, E, F).

in the cytoplasm of epidermal plates, cytons, and connecting bridges (Drobysheva, 2006). Single free centrioles occurred more frequently than the mature basal bodies with rootlets (Fig. 2 A). The basal bodies with rootlets and intracellular axonemes were positioned only in the cytoplasm of epidermal plates (Fig. 2 B, C). The intracellular axonemes were observed mainly in their apical parts (Fig. 2 C). In *G. wagini*, cilia were assembled on the basal bodies both in the cytoplasm of the epidermal layer and on the cell surface, following the docking of basal bodies to apical cell membrane (Fig. 2 B).

In the ventral epidermis of *G. intersticialis*, the overwhelming majority of centrioles were present as units consisting of basal body and rootlets, located in the epidermal plates (Fig. 3 A-C). In the cytons, centrioles were extremely rare. No intracytoplasmic axonemes could be seen in any of the epidermal cell compartments. Cilia assemblage started on the basal bodies, as soon as these basal bodies docked with the outer cell membrane (Fig. 3 A-C).

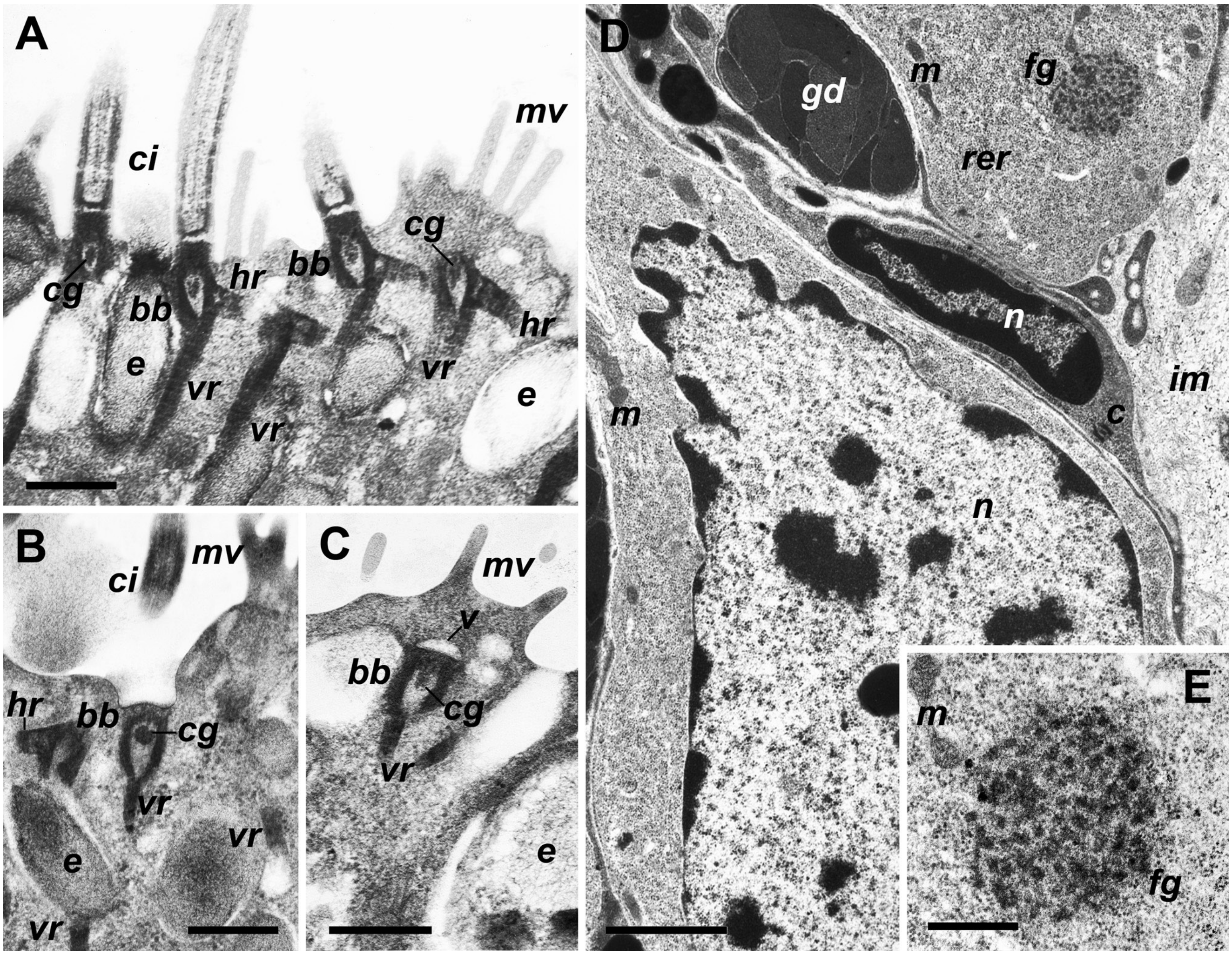

Fig. 3. – *Geocentrophora intersticialis*. Fragments of epithelial layer and cytons. (A, B, C) Parts of epidermal plates representing migration of basal body to cell surface. (A) Cilia on cell surface; free basal bodies with rootlets in cytoplasm. (B) Basal body anchored to cell membrane. (C) Basal body connected to a cytoplasmic vesicle. (D) Parts of two light and one dark cytons. (E) Cluster of fibrous granules in upper cyton (as on D) visible at higher magnification. bb, basal body; c, centriole; cg, central granule in centriolar cylinder; ci, cilia; e, epitheliosome; fg, fibrous granules; gd, parenchymal gland duct; hr, horizontal rootlet; im, intercellular matrix; m, mitochondria; mv, microvilli; n, nucleus of epidermal cell; rer, rough endoplasmic reticulum; v, cytoplasmic vesicle; vr, vertical rootlet. Scale bars = 0.5 µm (A–C, E), 2 µm (D).

Besides the above-described ciliary elements, small electron-dense fibrous granules, 50-80 nm in diameter, were also found in the ciliated epidermal cells of both *G. wagini* and *G. intersticialis*. In *G. wagini*, these fibrous granules generally formed clusters, sometimes containing up to several tens of fibrous granules (Fig. 2 D-F). The granules were not membrane-enclosed and their clusters were usually embedded in a fine granular matrix whose density was greater than that of the surrounding cytoplasm (Fig. 2 E, F). These fibrous granule clusters were noticeably more frequent in the cytons than in the epidermal plates of *G. wagini*. A very big cluster of fibrous granules was also found in one cyton of *G. intersticialis* (Fig. 3 D, E). Separate fibrous granules were observed near single centrioles or centriole groups in epidermal cells of both *G. wagini* and *G. intersticialis*. Some clusters of fibrous granules in *G. wagini* contained one to three centrioles or procentrioles (Fig. 2 F). This implied that fibrous granules either were built into centriolar cylinders as structural blocks or served only as a source of microtubule proteins, functioning as a microtubule organizing center (MTOC).

DISCUSSION

Two multiplication pathways for centrioles are distinguished during ciliogenesis: centriolar and acentriolar pathways (Anderson & Brenner, 1971). In the centriolar pathway, centrioles are generated in the contact with the parental centrioles. In the acentriolar pathway, centrioles are formed from centriole precursor structures without contact with preexisting centrioles. In the examined species centriologenesis clearly follows the acentriolar pathway. However, the ultrastructure of centriolar precursors is different between the species studied. In Lecithoepitheliata, centriolar precursors are fibrous granules usually gathered in clusters, while in Prolecithophora the precursor for each centriole is an individual filamentous accumulation. It is unclear if this difference is paralleled by variation in precursor molecular composition between two species.

EM-observations on ciliogenesis in other turbellarian taxa are rare and the available data suggest large variation in the pattern of centriologenesis among taxa. In the embryos of *Archaphanostoma* sp. (Acoela, Acoelomorpha), centriologenesis follows both classical and aberrant (in Bilateria) centriolar pathways (Tyler, 1984). In this acoel turbellarian, procentrioles arise not only on the centrioles and basal bodies, but also on the rootlets of established cilia. No centriolar precursors have been found during cilia development in the embryos of *Macrostomum hystricinum* (Macrostomida), where the procentrioles are generated "de novo" and by the centriolar pathway (Tyler, 1981). Clusters of centrioles are a prominent feature of the differentiating epidermal cells and epidermal stem cells in *Catenula lemnae*, *Stenostomum* sp., *Retronectes cf. sterreri* (Catenulida) (Soltynska et al., 1976; Ehlers, 1986). Early genesis of centrioles for cilia has been shown in the catenulid *Rhynchoscolex simplex* (Ehlers, 1992). In this species centriole multiplication starts in the M-phase during mitoses of intraepidermal stem cells. Both diplosomal centrioles are surrounded by many procentrioles concentrated in the cloud of centrosomal material from which astral and polar microtubules spread out (Ehlers, 1992).

All presently available data suggest that the mode of centriole formation and the ultrastructure of the centriolar precursors could be useful in reconstructing phylogenetic relationships within the Plathelminthes. Fibrous granules of *G. wagini* and *G. intersticialis* (Lecithoepitheliata) are morphologically identical to dense granules in differentiating epidermal cells of *Syndesmis echinorum* and *Paravortex cardii* (Rhabdocoela) (Cifrian et al., 1992). This common trait of ciliogenesis (a possible synapomorphy for Lecithoepitheliata and Rhabdocoela) could support joining taxa Lecithoepitheliata and Rhabdocoela in a single monophyletic group on some phylogenetic trees (Baguñà & Riutort, 2004). Another argument to consider characteristics of ciliogenesis as phylogenetically significant is the acentriolar mode of centriologenesis observed in Lecithoepitheliata, Rhabdocoela, and Prolecithophora and presumably lacking in Acoela, Catenulida, and Macrostomida.

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The pattern of FMRFamide and serotonin immunoreactive elements in the nervous system of *Aspidogaster conchicola* K. Baer, 1827 (Aspidogastrea, Aspidogastridae)

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ABSTRACT. The patterns of the neuropeptide FMRFamide and serotonin (5-HT) immunoreactive (IR) elements in the nervous system of Aspidogaster conchicola (Aspidogastrea, Aspidogastridae) are described using immunocytochemistry and confocal scanning laser microscopy. Both FMRFamid and 5-HT immunoreactivities occur in the bilobed brain, the three pairs of longitudinal nerve cords and many transverse commissures. The adhesive disc is strongly innervated by FMRFamide-IR nerve fibres. Many 5-HT-IR neurones were observed on the reproductive organs.

KEY WORDS: *Aspidogaster conchicola*, nervous system, FMRFamide, serotonin

INTRODUCTION

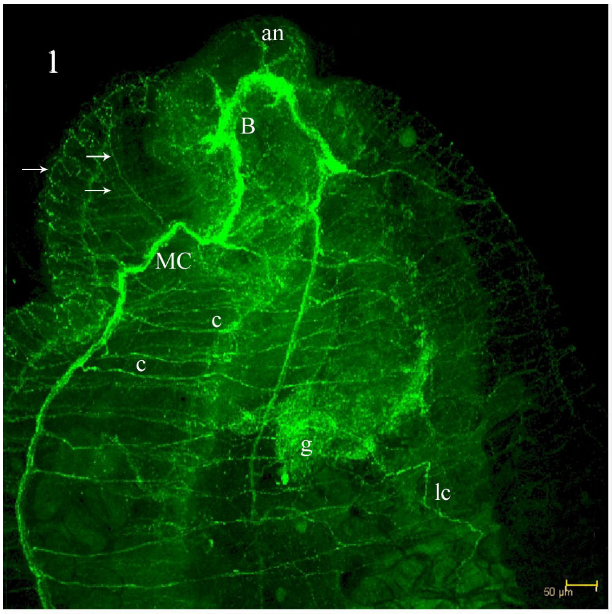
Olson & Tkach (2005) have recently put together a comprehensive review about the molecular systematics of parasitic Platyhelminthes. According to them Class Trematoda is composed of two subclasses, the Aspidogastrea comprising only a small number of species and the Digenea comprising about 18,000 nominal species. According to Schludermann et al. (2005) the aspidogastreans are of great importance as a model in evolutionary studies of parasitic flatworms, i.e., they form a possible link between free-living and parasitic organisms. The aspidogastreans have many ancestral features such as 1) a direct life cycle without larval multiplication, 2) low host and organ specificity, 3) an extremely complicated nervous system, 4) a very great number and variety of sensory receptors (Rohde, 2001). The aspidogastreans are less modified for a parasitic way of life than other Neodermata groups and they can survive several weeks outside a host animal. They lack the ventral sucker of the digeneans. Instead they have an adhesive disc (Baer's disc) on the ventral surface. The adhesive disc is divided into longitudinal rows of alveoli. The aspidogastreans are found in molluscs, fresh water and marine fish and sea turtles.

Timofeeva (1971) has given a detailed description of the cholinergic nervous system (NS) of *Aspidogaster conchicola*. In general the NS of A. conchicola resembles the NS of digeneans consisting of a distinct orthogon. *A. conchicola* lacks the thick nerve plexus found in monogeneans and cestodes (Kotikova, 1971). Shishov (1991) described the patterns of catecholamines and indolamines in *A. conchicola*.

As far as we know nothing is known about the peptidergic NS of *A. conchicola*. In the present study the patterns of neurons and nerve fibres immunoreactive (IR) to FMRFamide in *A. conchicola* are described and compared to those of serotonin (5-HT). To obtain a better picture of the spatial relationships between different parts of the NS and the body musculature, the anti-FMRFamide and anti-5-HT antibodies were used in combination with phalloidin-TRITC staining, which stains F-actin filaments.

MATERIALS AND METHODS

Specimens of adult *Aspidogaster conchicola* K. Baer, 1827 were recovered from the pericard of bivalves (*Anodonta anatina*) from the river Volga, (Tver Region, Russia). The material was fixed in 4% paraformaldehyde in 0.1M phosphate buffer at 4°C. For storage, it was transferred to the same buffer with 10% sucrose. Whole mounts of A. conchicola were stained with rabbit-anti-FMRFamide (Peninsula, Belmont, CA, USA) (1:500) or rabbit anti-5-HT (Incstar, Stillwater, MN, USA) (1:5000) according to the method described by Coons et al. (1955). The whole mounts were incubated with the primary antibody for seven days at 8°C and with the secondary antibody swine anti-rabbit FITC (DAKO) 1:50 for 5 days at 8°C. Controls included omission of the primary antibody and substitution of primary antibody with non-immune rabbit serum. Unfortunately double staining with anti-FMRFamide and anti-5-HT was not performed. In order to study the relationship between the patterns of the FMRF-IR and 5-HT-IR nervous elements and the musculature, staining with TRITC-conjugated phal-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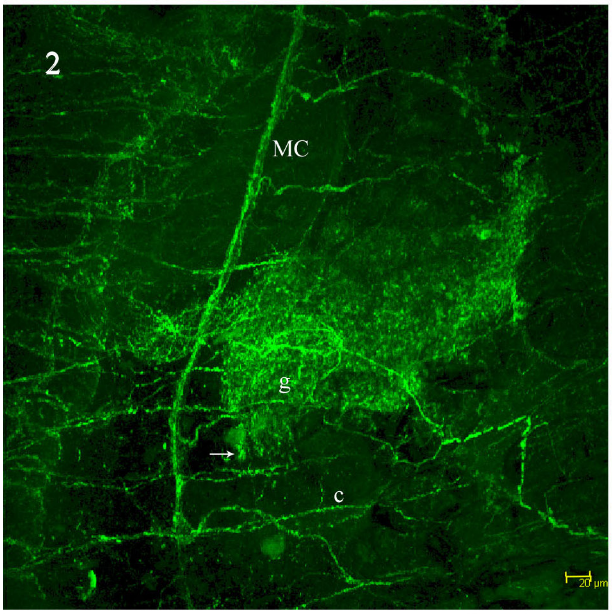
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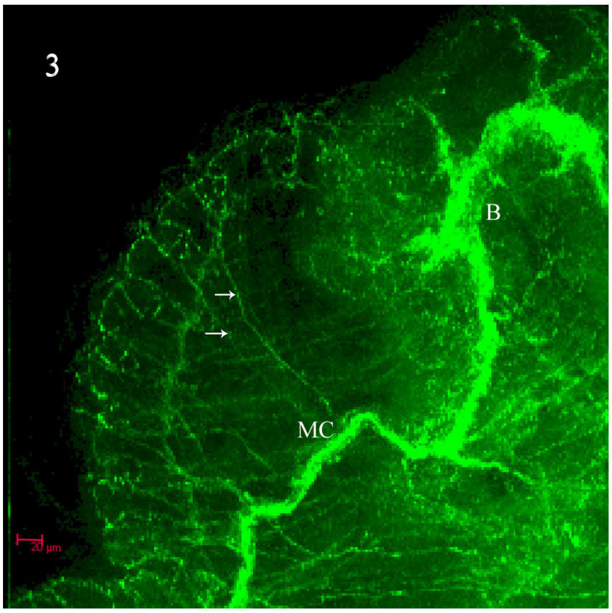
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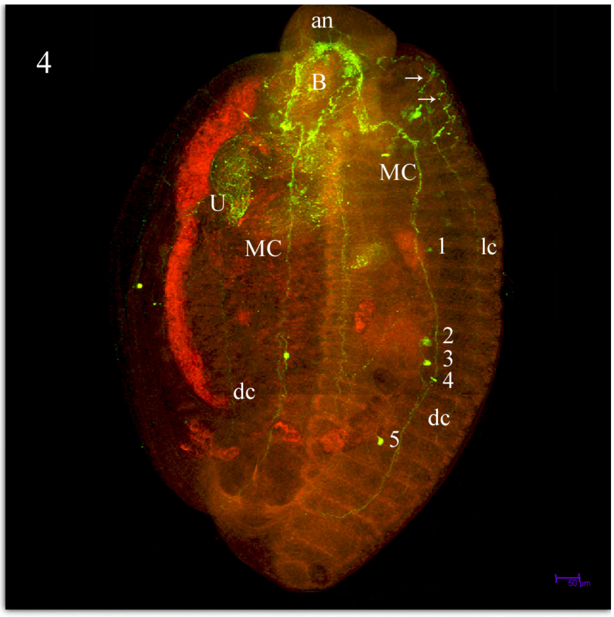
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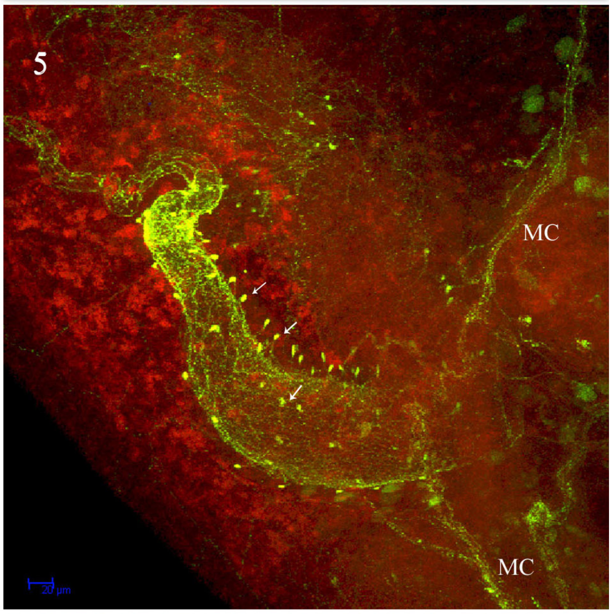
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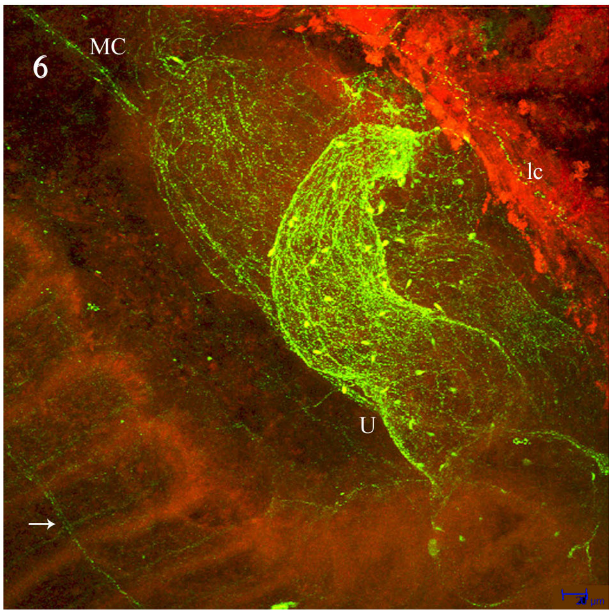
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loidin (Sigma, St. Louis, MO, USA) (1:200) was performed for 20min at 8°C (Wahlberg, 1998). The phalloidin staining was performed after the ICC staining. The ICC and the phalloidin stainings were performed at the Department of Biology, Åbo Akademi University, Finland.

RESULTS

Pattern of FMRFamide immunoreactivity

The plan of the flatworm NS is the so-called orthogon, a rectilinear, ladder-like configuration of longitudinal nerve cords connected at intervals by transverse ring commissures. The NS of *A. conchicola* follows this pattern. The staining with anti-FMRFamide is strong (Fig. 1). The bilobed brain is arch-shaped and measures about 25µm x 300µm. Many FMRFamide-IR nerve fibres extend from the brain in the anterior direction. They form a so-called terminal nerve ring close to the mouth. Three pairs of longitudinal nerve cords extend from the brain to the posterior end of the worm. The two ventral cords are the most prominent and will be called the main cords (MCs). The pairs of lateral and dorsal cords are thinner than the MCs. The longitudinal cords are connected by many transverse ring commissures. The FMRFamide-IR neurones are small and difficult to count. In the region of the genital opening an FMRFamide-IR nerve net including one neurone was observed (Fig. 2). Numerous FMRFamide-IR fibres innervate the adhesive disc (Fig. 3). Many FMRFamide-IR fibres were observed in the subepithelial nerve plexus and surrounding the excretory pore.

Pattern of 5-HT immunoreactivity

The staining with anti-5-HT was weaker than that of anti-FMRFamide (Fig. 4). 5-HT immunoreactivity was observed in the arch-shaped bilobed brain, in fibres extending in the anterior direction forming the so-called terminal ring at the edge of the mouth, the two MCs, the pairs of dorsal and lateral longitudinal cords extending to the posterior end of the worm. Five 5-HT-IR neurones (size 10 x 15 µm) were observed in the brain, two in the anterior nerve fibres and one in the terminal nerve ring. Five 5-HT-IR neurones (size 10x15µm) occur along each MC. The longitudinal cords are connected by a few transverse commissures. Many 5-HT-IR fibres were observed in the subepithelial nerve plexus. Close to the genital opening one 5-HT-IR neurone was observed. Numerous 5-HT-IR neurones (size 4 x 5 µm) and fibres were observed on the surface of the uterus (Figs 5-6). A few 5-HT-IR neurones were observed in connection with the distal parts of the male reproductive system. Four longitudinal and several transverse muscular septa divide the adhesive disc into numerous discrete alveoli. A nerve net composed of thin 5-HT-IR fibres was found inside the adhesive disc (Fig. 6).

DISCUSSION

As expected the NS of *A. conchicola* contains peptidergic (FMRFamide-IR) and 5-HT-IR nervous elements. The presence of cholinergic, catecholaminergic and indolaminergic substances in *A. conchicola* had previously been demonstrated by Timofeeva (1971) and Shishov (1991). This means that *A. conchicola*, with reference to the main categories of neuronal mediators, resembles all other flatworm species so far examined. The presence of acetylcholine (ACh), FMRFamide- and 5-HT-IR innervation of the mouth, adhesive disc and reproductive system indicates that the above-mentioned neuronal mediators take part in the regulation of the muscle activity in these vital parts. A physiological role for ACh as an inhibitory neurotransmitter in most trematodes and cestodes is indicated by the fact that their muscular activity is reduced by cholinomimetics and by cholinesterase inhibitors, ultimately producing a flaccid paralysis. 5-HT appears to be the dominant biogenic amine in all flatworm species examined, and there is good experimental evidence that it serves a variety of functions, most notably that of excitatory neurotransmition (Gustafsson & Halton, 2001). The function of neuropeptides such as FMRFamide-like peptides (FLPs) in flatworms is as yet unclear but their ubiquitous occurrence throughout the flatworm NS implies a fundamental role in the nerve-muscle physiology. All FLPs so far isolated and sequenced from flatworms have been shown to be myoexcitatory in a concentration-dependent manner when applied exogenously to living muscle cells or muscle strips from free-living and parasitic flatworms (McVeigh et al., 2005).

In 1998 Reuter et al. introduced new concepts into the flatworm neurobiology. The terminology of the longitudinal nerve cords had long been very confusing. In order to obviate these difficulties, two new terms were coined, the ‘main

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Fig. 1-3. – The pattern of FMRFamide-IR nerve fibres in *Aspidogaster conchicola.*
Fig. 1. – Anterior part of the animal. Arrows: innervation of the adhesive disc
Fig. 2. – The innervation of the reproductive organs. Arrow: neurone.
Fig. 3. – The innervation of the adhesive disc. Arrows: nerves from the MC to the adhesive disc.

Figs 4-6 – The pattern of 5-HT-IR nerve fibres in *Aspidogaster conchicola*.
Fig. 4. – neurones along the MC indicated with 1, 2, 3, 4, 5; arrows: nerves to adhesive disc.
Fig 5. – The innervation of the reproductive organs. Note many small 5-HT-IR neurones (arrows) at the surface of the uterus.
Fig. 6. – The innervation of the adhesive disc. 5-HT-IR nerves form a regular network in the adhesive disc. Many small 5-HT-IR neurones on the wall of uterus. Arrow: innervation of the adhesive disc.

Abbreviations: B: bilobed brain; an: anterior nerves; c: transverse commissures; dc: dorsal nerve cord; g: genital pore; lc: lateral nerve cord; MC: main nerve cord; U = uterus.

nerve cords' (MCs) and the 'minor nerve cords'. The MCs are defined as the two most prominent nerve cords in the worm. Irrespective of their disposition as ventral, dorsal or lateral, the MCs originate as multifibre outgrowths or rootlets from each of the brain ganglia and are associated with more neurones that any other nerve cord. The minor cords comprise all other longitudinal cords. The concept of MCs provides for the possibility of dividing the flatworm NS into a central nervous system (CNS) and a peripheral nervous system (PNS). The CNS comprises the bilobed brain and the MCs; the PNS comprises all of the minor cords and the nerve plexuses. The ventral nerve cords of *A. conchicola* fill the criteria for MCs and make it possible to divide the NS into a CNS and a PNS.

In order to establish their phylogenetic position, the aspidogastreans have been investigated from many different angles, from light, confocal scanning laser, transmission and scanning electron microscopy to molecular biology (Rhode, 1994, 2001, 2002; Levron et al., 2009; Gao et al., 2003; Olson & Tkach, 2005). Raikova (2004) investigated the neuroanatomy of basal bilaterians (Xenoturbellida, Nemertodermatida, Acoela) and its phylogenetic implications. She pointed out that "the NS characters seem very useful to unravel the acoel taxonomy at a low level (family, genus and species), within a closely related monophyletic group. The use of NS characters at a higher level may be misleading, because of a high degree of homoplasy and the fact that the NS evolution has taken place independently and in parallel in many monophyletic lines and not within Acoela as a whole." This means that the ICC results obtained in this study have no implications for the discussion of the phylogenetic position of the aspidogastreans but they still form a piece in the general jigsaw puzzle of the flatworm NS.

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Characterization of *hsp* genes in planarian stem cells

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ABSTRACT. Planarians are a model system known for regenerative potential, body plasticity and continuous turnover of all differentiated cell types. These characteristics are based on the presence of pluripotent stem cells, called neoblasts. Damage or reduction in the number of neoblasts deeply affects planarian regeneration and survival. Heat shock proteins (HSPs) are known to perform essential cytoprotective functions in all organisms. To investigate the potential role of *hsp*-related genes on the dynamics of planarian stem cells, representative *hsp*-related genes were identified and characterized in normal conditions and after different stress stimuli. Our work revealed that two different *hsp* genes (*Djhsp60* and *Djmot*) are constitutively expressed in neoblasts, suggesting that their products play important roles in cytoprotection of these cells. RNAi-based functional studies provide evidence of an involvement of *Djhsp60* and *Djmot* in the adaptive response of planarian stem cells to stress and indicate that expression of these genes is critical for planarian survival.

KEY WORDS: *Dugesia japonica*, neoblasts, regeneration, heat shock genes, RNAi

INTRODUCTION

Stem cells are crucial to homeostasis and regeneration of all metazoans (Rando, 2006; Pellettieri & Sánchez Alvarado, 2007). High stress tolerance concomitant to an increased expression of Heat Shock Proteins (HSPs) in stem cells has recently attracted attention, suggesting that HSP members are fundamental to modulate behavior, prevent senescence and prolong proliferative capacity of these cells (reviewed in Prinsloo et al., 2009). HSPs can be detected in all organisms and form the most ancient defence system. The heat shock response was first identified in the salivary glands of *Drosophila melanogaster* upon application of a transient exposure to elevated temperatures (Ritossa, 1962). Later, more functions involving HSPs were uncovered, indicating HSPs as the major regulatory proteins in the cell (Sreedhar & Csermely, 2004). HSPs are functionally related proteins classified into families according to molecular weight. Within each class there are members that are constitutively expressed or finely regulated, and/or specific of different compartments (Prinsloo et al., 2009). Although HSPs are highly expressed in stressed cells and could be considered only as cell stress “buffers”, they are also involved in gene expression regulation, DNA replication, signal transduction, differentiation or immortalization (Jolly & Morimoto, 2000). Various studies demonstrate that HSP-induced cytoprotection promotes cell survival by an important contribution to the signals directing the cell to senescence, apoptosis, or necrosis. These proteins can be also involved in immune response stimulation (Sõti et al., 2003; Sreedhar & Csermely, 2004).

Planarian flatworms - well known for regenerative potential and body plasticity - represent a unique model system to study adult stem cells. Planarian stem cells, named neoblasts, are pluripotent cells continuously recruited to replace aged differentiated cells, and allow regeneration in these organisms (recently reviewed by Pellettieri & Sánchez Alvarado, 2007; Handberg-Thorsager et al., 2008; Rossi et al., 2008). The long lifespan of planarians, coupled with the possibility to analyze *in vivo* the behavior of their stem cells during tissue homeostasis and regeneration, provides a unique opportunity for understanding how stem cells respond to stress in more complex metazoans, including humans. Damage or reduction in number of neoblasts deeply affects planarian regeneration and survival. To investigate the potential role of HSP-related genes on the dynamics of neoblasts, we characterized representative HSP-related genes in normal conditions and after different stress stimuli and identified two neoblast-specific HSP members, *Djhsp60* and *Djmot*, belonging to the HSP60 and HSP70 gene families, respectively (Rossi et al., 2007; Conte et al., 2009). Both these genes are involved in the adaptive response of planarian stem cells to stress conditions. *Djhsp60* or *Djmot* RNAi-mediated functional ablation causes growth arrest in neoblasts. The possibility that DjMot plays an essential role in a conserved mechanism of cytoplasmic sequestration of p53, thus antagonizing its nuclear entry, is discussed.

MATERIALS AND METHODS

Asexual specimens of *D. japonica* (GI strain) were maintained at 18°C in autoclaved stream water, fed weekly with chicken liver and used for experiments after 10 days of starvation. Thirty days-starved planarians were used for starvation analysis. Regenerating fragments were produced by transverse amputation. Some intact worms were exposed to a lethal dose (30Gy) of X-rays as described by Conte et al. (2009). For heat shock treatment, intact planarians were maintained o/n at 28°C before harvesting for RNA extraction. In situ hybridization and RNAi were performed as described by Conte et al. (2009). Total RNA for real time reverse transcription (RT)-PCR experiments was extracted from three planarians, in triplicate, with the NucleoSpin RNAII kit (Macherey and Nagel). Each extraction was tested for the absence of genomic DNA by amplifications performed in the absence of reverse transcriptase. Superscript First Strand Synthesis System kit (Invitrogen) was used for cDNA synthesis. SYBR Green chemistry-based reactions were carried out as described by Conte et al. (2009) with a Rotor-Gene 6000 (Corbett Research). The mRNA levels of specific genes were compared with controls using planarian *elongation factor 2 (Djef2)* as reference gene to normalize RNA input (Rossi et al., 2007). The primer sets used in the experiments, generated using NetPrimer software, were as follows:

Djhsp60 forward primer:
5'TATTGTCGCATCGTTGAAAGC3';
Djhsp60 reverse primer:
5'CCAATTCATCATGTAATGTTTT3';
Djmot forward primer:
5'GCATTCCACCAGCACCTC3';
Djmot reverse primer:
5'CATATTTTCAATTTCATCTTTACTCAA3'.
Djef2 forward primer:
5'CAATCGAAGACGTTCCATGTG3'
Djef2 reverse primer:
5'AACACGAACAACAGGACTAAC3'

RESULTS

Based on the analysis of a *Dugesia japonica* EST collection (Mineta et al., 2003) we identified some cDNAs coding for HSP members with different molecular mass. In particular, several cDNA fragments coding for HSP70-related proteins were found. In order to distinguish more precisely the number of HSP*70*-related genes in *D. japonica*, several couples of primers were designed to amplify cDNA regions included between the different gene fragments (data not shown). RT-PCR analysis demonstrated that at least three HSP*70*-related genes exist in planarians. Two of these genes code for very similar constitutive HSP70 forms (HSC70), while the third gene encodes a peculiar HSP70 member showing high similarity with mammalian Mortalin (*Djmot*: Conte et al., 2009). One of the two HSC70 appeared to be identical at the nucleotide level to *Djhsc70* (accession number ABY83101). In situ hybridization experiments revealed that the HSC70 genes were ubiquitously expressed (not shown), while *Djhsp60* (EST 32903936: Mineta et al., 2003) and *Djmot* had a pattern similar to that shown by other neoblast-specific genes (for example, *Djmcm2*: Salvetti et al., 2000) (Fig.1 A-F). Both *Djhsp60* and *Djmot* parenchymal expression was strongly downregulated in animals exposed to a lethal dose of X-ray (30Gy) (a treatment that destroys neoblasts: Hayashi et al., 2006; Rossi et al., 2007; 2008; Conte et al., 2009) while other *hsp* genes were not affected (not shown). Different stress conditions, such as heat shock (28°C o/n) or a long period of starvation, strongly activated *Djhsp60* transcription (Fig.2 A,B), while *Djmot* upregulation was only observed after prolonged starvation (Conte et al., 2009).

Expression profile of *Djhsp60* and *Djmot* during regeneration was analyzed by in situ hybridization. Regenerating fragments showed strong upregulation of *Djhsp60* transcripts in the blastema, the postmitotic area where neoblast progeny undergo differentiation, and in the stump region beneath the blastema (postblastema), comprised of actively proliferating neoblasts. This accumulation, clearly detected by 2 days after amputation, declined from the regenerating area as regeneration proceeded (Fig.3 A-H).

Djmot transcription appeared upregulated in the postblastema, and a certain level of hybridization signal could be detected also in the blastemal tissue by 2 days after amputation. However, after 4 days, *Djmot* expression was completely absent from the blastema and declined in the postblastema to the normal levels observed in intact animals (Fig. 3 I,J). These results suggest that *Djmot* transcripts are present in proliferating neoblasts, and residual expression may transiently remain in early postmitotic progenitors (Conte et al., 2009).

To assess whether *Djhsp60* and *Djmot* play a role in neoblasts, we performed sequence-specific gene silencing by double strand(ds)RNA-mediated interference. Similarly to that observed after *Djmot* RNAi (Conte et al., 2009), functional inhibition of *Djhsp60* caused tissue regression in intact planarians, possibly indicative of homeostatic defects (Fig. 4 A,B). However, only 15% (23/150) among the *Djhsp60* dsRNA-injected specimens showed a morphologically detectable phenotype, in spite of the consistent reduction of the level of *Djhsp60* endogenous transcripts (not shown).

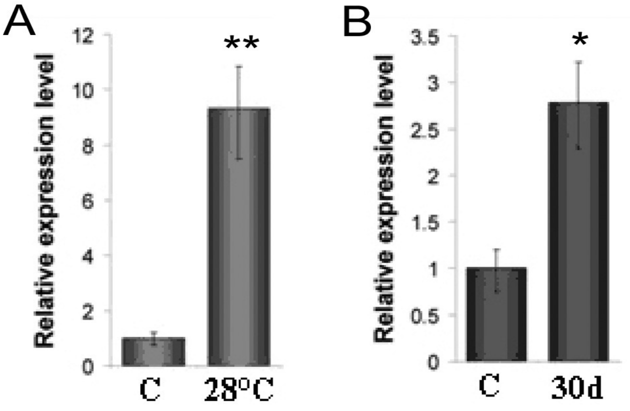

Fig. 2. – ***Djhsp60* expression level in different stress conditions, visualized by real time reverse transcription (RT)-PCR.** (A) heat shock (28°C) (B) 30 days of starvation. d: days of starvation. Expression levels are indicated in relative units, assuming the value of the untreated specimens (C: control) as unitary. Each value is the mean±s.d. of three independent samples, analyzed in triplicate. Samples were compared using the un-paired t-test.**P<0.001, *P<0.05.

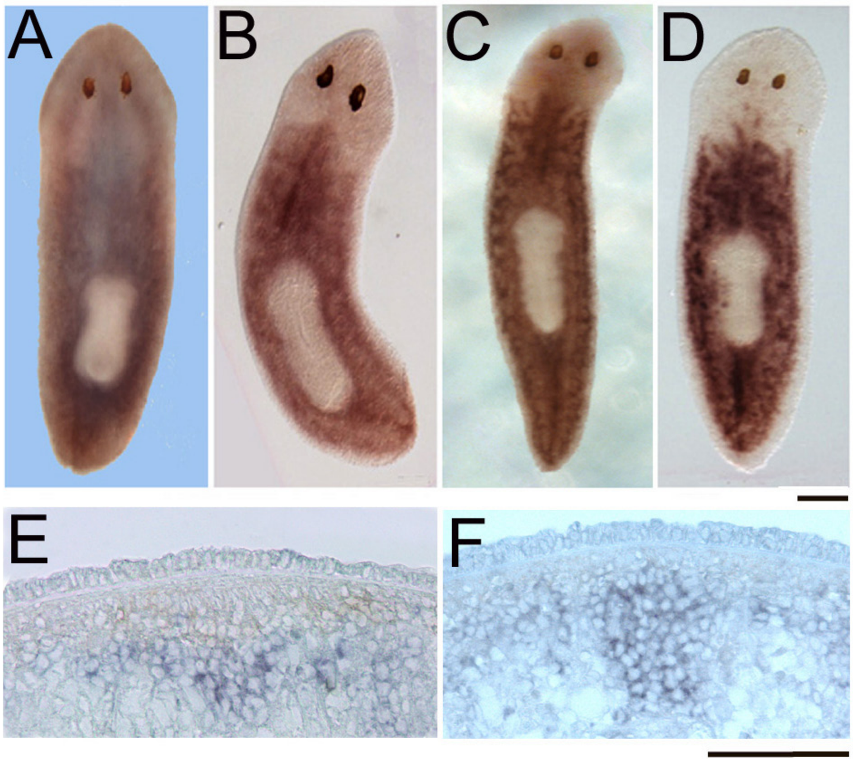

Fig. 1. – **Expression pattern of *hsp* genes in *D. japonica*.** (A-D) Whole mount in situ hybridization on intact planarians. (A) *Djhsc70*; (B) *Djhsp60*; (C) *Djmot*; (D) *Djmcm2* is shown for comparison. (E,F) In situ hybridization on transverse sections, visualized by NBT/BCIP chromogen precipitation in small neoblast-like cells of the dorsal midline region at the prepharyngeal level. (E) *Djhsp60*. (F) *Djmot*. Scale bars: 500 µm in A-D, 100 µm in E,F.

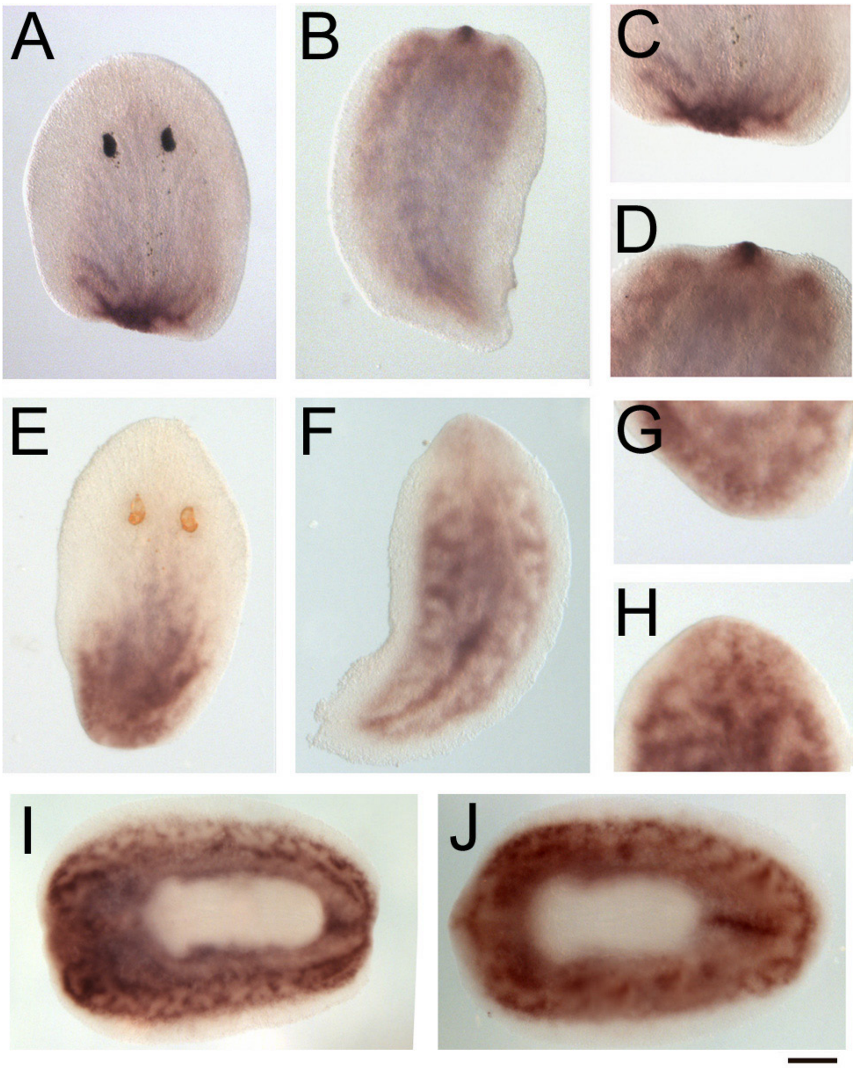

Fig. 3. – **Expression pattern of *Djhsp60* and *Djmot* during regeneration, visualized by whole mount in situ hybridization.** (A-H) *Djhsp60*. (A) posterior and (B) anterior regeneration, 2 days of regeneration. (C) Enlarged view of the regenerating area depicted in A. (D) Enlarged view of the regenerating area depicted in B. (E) posterior and (F) anterior regeneration, 4 days of regeneration. (G) Enlarged view of the regenerating area depicted in E. (H) Enlarged view of the regenerating area depicted in F. Anterior is on the top. (I,J) *Djmot*. (I) A trunk fragment regenerating both a head and a tail, 2 days of regeneration. (J) A trunk fragment regenerating both a head and a tail, 4 days of regeneration. Anterior is on the left. Scale bars: 50 µm in A, B, E, F, I, J; 100 µm in C, D, G, H.

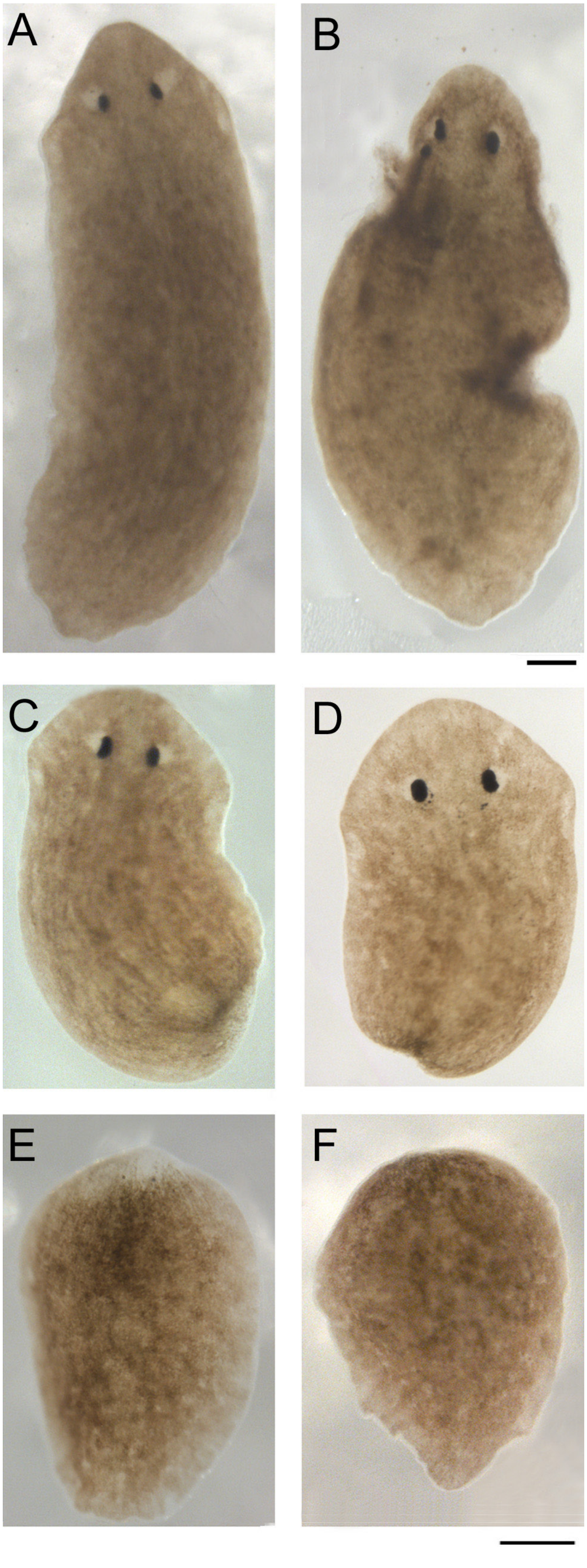

Fig. 4. – **Analysis of *Djhsp60*RNAi phenotypes.** (A,B) Bright-field images of intact planarians, 20 days after the first injection. (A) A water-injected control. (B) A *Djhsp60*RNAi phenotype. (C-F) Bright-field images of regenerating planarians. (C,D) posterior and (E,F) anterior regeneration, 5 days after amputation. (C) A water-injected control. (D) A *Djhsp60*RNAi phenotype unable to regenerate. (E) A water-injected control. (F) A *Djhsp60*RNAi phenotype unable to regenerate. Anterior is on the top. Scale bars: 300 μm.

When amputated, only a limited number of injected fragments did not form a regeneration blastema (20%, 20/100, from three independent experiments) (Fig. 4 C-F). The majority of *Djhsp60* RNAi specimens were able to regenerate, although some of them exhibited a smaller blastema.

Conversely, *Djmot* RNAi strongly inhibited blastema formation and most of the injected fragments completely failed to regenerate. In intact planarians *Djmot* knockdown did not produce any gross morphological defect. All injected specimens (both intact animals and amputated fragments), however, started to die in 4-5 weeks after the first injection, while the survival of controls was not affected (Conte et al., 2009). As it has been demonstrated that HSP60 may be functionally associated with mitochondrial HSP70 (mtHSP70/Mortalin) (Deocaris et al., 2006; Wadhwa et al., 2005) we also coinjected an equimolar mixture of *Djmot* and *Djhsp60* dsRNA. The results did not show any significant variation either in the type or the number of phenotypes (not shown), suggesting that the activity of these two genes in neoblasts involves different mechanisms. In addition, we observed that the levels of *Djhsp60* transcripts were not affected by *Djmot* RNAi (not shown).

DISCUSSION

Djhsp60

Djhsp60 is expressed in the parenchyma in small neoblast-like cells that are specifically eliminated by X-ray irradiation. This finding confirms the data obtained by Rossi and coworkers who found selective *Djhsp60* down-regulation after X-ray treatment (Rossi et al., 2007). However, in this work we have observed that a *DjHSP60*-mediated stress response is specifically activated in postmitotic progenitors during regeneration. *Djhsp60* expression was in fact strongly upregulated in the early blastema, a region devoid of mitotic activity. The loss of body parts probably subjects the remaining cells to a number of stresses, and HSP60 is a chaperone involved in the appropriate folding and assembly of polypeptides into protein complexes. Literature data demonstrate that wounding can induce specific HSP60 response, indicating that appropriate stress response processes may be correlated with regenerative success also in other organisms (Laplante et al., 1998; Makino et al., 2005). Recent studies during limb regeneration in newts reveal that high apoptosis levels are present in the first days after amputation, while a few apoptotic cells are detected one week post-amputation (Vlaskalin et al., 2004). Our data support the possibility that upregulation of *Djhsp60* transcripts may play a role in preventing programmed cell death in order to protect integrity of the new tissue during the early regeneration events. *Djhsp60* induction after heat shock or starvation further supports the possible cytoprotective role of HSP60 in planarians. *Djhsp60*RNAi experiments also indicated that induction of this gene may be required for appropriate regeneration. However, the overall percentage of abnormal phenotypes observed in *hsp60* RNAi animals was very low, probably due to redundant effects of other *hsp60*-related genes. Although the presence of other *hsp* transcripts may compensate for the loss of *Djhsp60* function, we cannot completely exclude that DjHSP60 plays only an auxiliary role in the regulation mechanisms involved in survival/ maintenance of planarian cells, including stem cells (Patterson & Klingenberg, 2007).

Djmot

Djmot is a planarian gene that encodes a protein showing high identity with a heat-uninducible member of the mammalian HSP70 family, identified as Mortalin. In physiological conditions high levels of *Djmot* transcripts are detected in proliferating neoblasts and their descendants, while no detectable expression of this gene can be found in differentiated cells (Conte et al., 2009). Literature data demonstrate that Mortalin-like proteins are essential for cell viability in different organisms, such as yeast, *C. elegans* and mammals (Kimura et al., 2007; Wadhwa et al., 2002; Wadhwa et al., 2005). The phenotypes associated with *Djmot* RNAi also indicate an involvement of this gene in maintaining neoblast viability (Conte et al., 2009). Recent work demonstrates that Mortalin-like proteins may sequester the tumor suppressor protein p53 in the cytoplasm, preventing senescence and apoptosis, and thus promoting lifespan and immortalization of cells in different organisms (Sherman et al., 2007; Wadhwa et al., 2002; Walker et al., 2006). The molecular events that allow neoblasts to escape cellular senescence remain undefined so far. Consistent with the possibility that *Djmot* is necessary to prevent growth arrest in dividing stem cells of planarians, a variable number of flattened, enlarged cells with condensed chromatin were detected in dissociated cells of *Djmot*RNAi planarians (Fig. 5 A,B). This type of cell was never observed in control animals and we suggest that these cells could be senescent cells. Unfortunately, no biomarkers for senescent cells are available in planarians. It is well known that two tumor suppressor proteins, p53 and Rb, play a crucial role in the senescence response (Campisi, 2005). We are tempted to speculate that the relationship between *Djmot* functional ablation and the induction of senescent cells could be related to ability of planarian Mortalin to function as a buffer of a planarian p53-like protein, modulating its activity. Functional inhibition of *Djmot* would allow release of p53 into the nucleus and consequent activation of cell senescence programs (Fig. 5 C,D). Apoptosis is widely cited as the primary mode of stem cell deletion during adult tissue homeostasis. However, it is very difficult to investigate how stem cells die in planarians. Recent investigation of cell death in planarians, including TUNEL assay, yielded ambiguous results (Pellettieri & Sánchez Alvarado, 2007). Our preliminary results, obtained by a Comet Assay protocol adapted for planarians (Prá et al., 2005), provided evidence that, after *Djmot* RNAi, about 10-20% nuclear comets could be detected, a percentage that reflects the presumptive number of neoblast-like cells in planarians (Baguñà & Romero, 1981). Further studies, including characterization of *p53* genes, are needed to further elucidate the molecular pathways implicated in growth control of planarian stem cells.

Conclusions

The molecular characterization of the planarian genes *Djhsp60* and *Djmot* reveals a first insight into the complex scenario of the stress response in planarians and, in particular, in the analysis of genes involved in the protection of the pluripotent stem cell system of these organisms. Certainly, *hsp60* induction seems to be a shared requirement for regeneration of body parts in vertebrate and invertebrate organisms. However it is still unknown whether this gene product plays a general role in cellular stress or, under traumatic circumstances, can be released from cells to regulate immune or inflammatory responses (Calderwood et al., 2007). Conversely, a fundamental and conserved role of Mortalin-like proteins appears to be the modulation of p53 activity in immortalized or stem cells, thus preventing senescence and

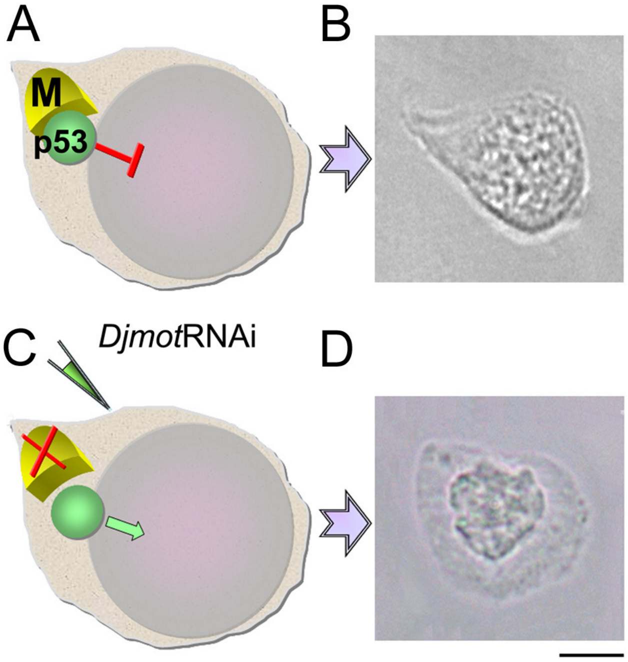

Fig. 5. – **Hypothetical model for DjMot function in neoblasts.** (A) DjMot (M) prevents nuclear translocation of p53–like protein**.** (B) Phase contrast image of a neoblast. (C) *Djmot*RNAi disrupts DjMot-p53 interaction and allows nuclear translocation of p53. (D) Phase contrast image of a senescent cell, as detected after *Dmot*RNAi. Scale bar: 5μm.

prolonging proliferative capacity of these cells (Prinsloo et al., 2009). Further investigation will be essential to identify conserved mechanisms that regulate both stress response in adult stem cells and injury-induced epimorphic regeneration of different organisms.

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Planarian regeneration in the absence of a blastema

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ABSTRACT. During planarian regeneration, a new tissue called the blastema is formed after amputation and is thought to play an important role in pattern formation. To investigate its role, posterior fragments with irradiated stumps were generated by regional X-irradiation, followed by amputation. No blastema formation occurred in the irradiated fragments until 3 days after amputation, because all the neoblasts had been depleted from the stump area by irradiation. However, regeneration of the pharynx occurred in the predicted region of the fragment, even in the absence of a blastema. These results suggest that the blastema is not essential for pattern formation during planarian regeneration.

KEY WORDS: pattern formation, polarity, intercalary regeneration, neoblasts, platyhelminthes

INTRODUCTION

Freshwater planarians possess many organs, including a central nervous system, a complicated branched gut, a pharynx and protonephridial ducts. Planarians have a defined body pattern and each of these organs is located in a specific position (Hyman, 1951). Planarians also exhibit remarkable regeneration abilities; the head and tail can both regenerate if the anterior or posterior parts are cut off, respectively. Complete animals with body patterns the same as that of the original animal can be regenerated from tiny body fragments derived from any region posterior to the eyes, except the pharynx. When and how does body pattern formation occur during planarian regeneration? Regeneration is divided into several processes (Baguñà, 1998). Following amputation, (1) the wound is closed by extension of the epithelial cell layer, (2) a blastema is generated at the wound through the migration and proliferation of neoblasts in the stump below the wound, (3) the blastema grows by subsequent proliferation and migration of the neoblasts, and (4) cell differentiation occurs and new tissue is formed. What is a role of the blastema in these processes? Based on intercalary regeneration observed in grafting experiments, Agata et al. (2003) speculated that intercalation between the blastema and the old stump induced rearrangement of the body regions. According to a previous model, pattern determination occurs in a narrow strip (200–300 µm) of the stump below the wound, as a result of a distal-proximal sequence of induction during the first day of regeneration. The pre-patterning is subsequently amplified and translated into morphological patterning through cell proliferation and differentiation as the blastema grows (Baguñà, 1998). Thus, the blastema is thought to play an important role in pattern formation during planarian regeneration.

In this study, regional X-irradiation was used to produce regenerating fragments with no blastema. Regeneration of the pharynx was observed in the same position as in un-irradiated fragments. These results suggest that the blastema does not play an important role in pattern formation during planarian regeneration.

MATERIALS AND METHODS

The isogenetic asexual strain HI of the freshwater planarian *Dugesia japonica* was used in this study (Kato et al., 1999). Animals were maintained at room temperature (about 22^{o}C) in autoclaved tap water and fed with chicken liver once a week. Worms 1.5–2 cm in length were selected and starved for at least 4 days before use. Regional irradiation was performed as described previously (Ito et al., 2001; Orii et al., 2005). Briefly, worms were placed on wet filter paper on ice. The un-irradiated region was covered with a lead sheet, while the remainder was irradiated using a SOFTEX B-4 X-ray generator (Softex) (Fig. 1).

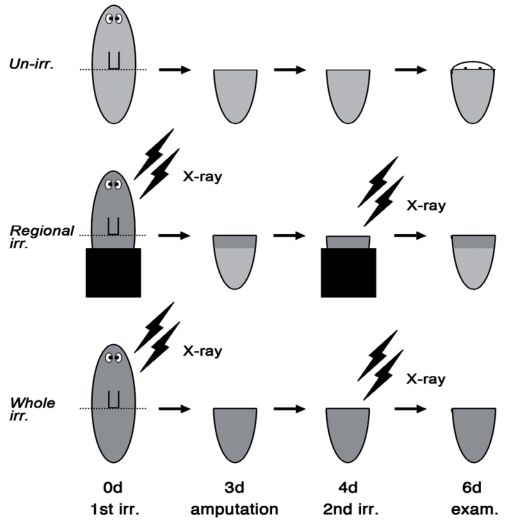

Fig. 1. – Diagram showing irradiation and amputation experiments. The posterior fragments from un-irradiated (upper), regionally irradiated (middle), and completely irradiated planarians (lower) were allowed to regenerate for 3 days. Blastemas developed in un-irradiated fragments, but not in completely or regionally irradiated fragments.

The radiation dose was about 55 R, which is the minimum dose required for complete inhibition of regeneration in this planarian strain (Kato et al., 2001). Three days after irradiation, the planarian was amputated, as shown in Fig. 1, and the posterior fragment was allowed to regenerate at room temperature. On the next day, the stump region was re-irradiated in the same way. The second regional irradiation was performed to deplete the neoblasts in the stump region completely, which resulted in many regenerating fragments with no blastema. Three days after amputation, the fragment was observed under a binocular microscope to confirm the absence of a blastema. The fragment was then fixed, embedded in paraffin, and sectioned, according to the standard protocol (Kobayashi et al., 1998). Sagittal sections were subjected to in situ hybridization using the myosin heavy chain-A *(Djimch-A)*, as a marker gene for the regenerating pharynx, as described previously (Kobayashi et al., 1998; Sakai et al., 2002).

RESULTS AND DISCUSSION

Freshwater planarians possess many pluripotent stem cells, called neoblasts, which account for about 30% of the total cell number (Baguna & Romero, 1981; Orii et al., 2005). Neoblasts are distributed in the mesenchyme throughout the body, except in the pharynx and the region anterior to the eyes (Orii et al., 2005). Neoblasts are sensitive to X-rays, and regional irradiation of planarians results in depletion of the neoblasts in the irradiated part. Regional irradiation was used about 50 years ago to investigate the contribution of neoblasts to planarian regeneration (Wolff, 1962). Using this method, we also previously demonstrated that the distal part of the pharynx was able to regenerate in an epimorphic manner (Ito et al., 2001).

In this study, posterior fragments with an irradiated stump were prepared by regional irradiation followed by amputation. The region where the pharynx would regenerate was not irradiated. Three days after amputation, a small pair of eyes was observable between the blastema and the stump in the fragments derived from un-irradiated planarians (Fig. 2A). As predicted, some fragments with an irradiated stump had no blastema (Fig. 2B). These were similar to fragments from completely irradiated planarians (Fig. 2C). Pharynx regeneration in the fragment with no blastema was examined by in situ hybridization using *Djmhc-A* gene. This gene is strongly expressed in pharynx muscle cells and in pharynx-anchoring muscles, and therefore provides a good marker of pharynx regeneration (Kobayashi et al., 1998; Orii et al., 2002). A rudimentary pharynx generally appears in the tail fragment 2–2.5 days after amputation, and then increases in size (Sakai et al., 2002). Surprisingly, a regenerating pharynx was observed at the normal position in the fragments with no blastema (Figs. 2B and E). In addition, the timing of the regeneration process appeared to be similar, or only slightly delayed, in comparison with that in un-irradiated fragments (Figs. 2D and E). No sign of pharynx regeneration was observed in completely irradiated fragments (Fig. 2F). These results indicate that the blastema is not required for normal pharynx regeneration in posterior planarian fragments.

Until now, the blastema has been thought to play a central role in pattern formation. This theory is based on the results of numerous grafting experiments; a graft from a different region induces new tissue between the graft and the surrounding tissue (Agata et al., 2003). It is possible that such induction occurs in a narrow stump below the wound (Baguna, 1998). However, there is no evidence that pattern formation during regeneration after amputation occurs in same way as intercalary regeneration after grafting.

Hox genes, which are expressed along the anteroposterior axis and are thought to play a role in maintaining body pattern, are also expressed in the posterior blastema. Their expression is concentrated in the posterior part of the posterior fragment after amputation (Orii et al., 1999). The

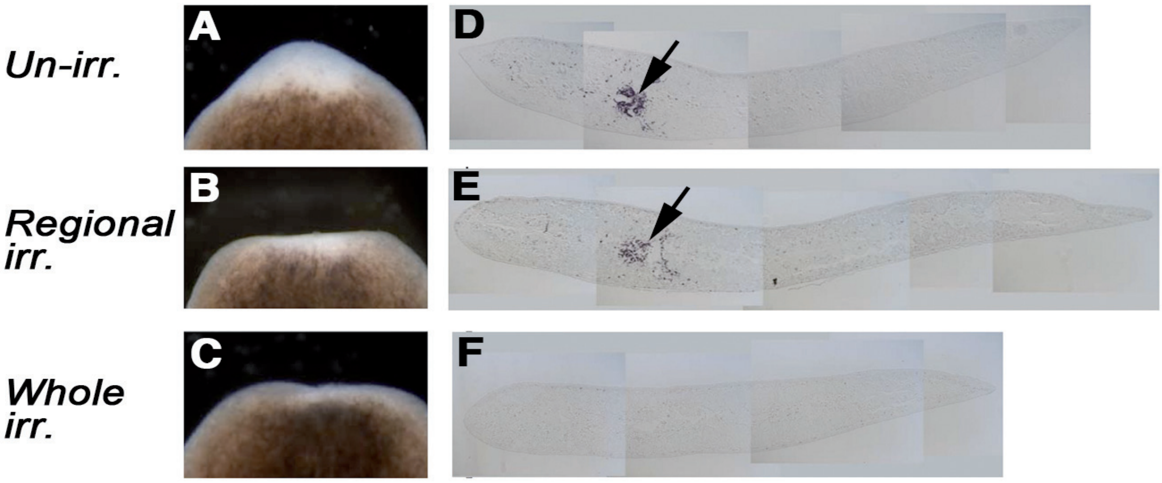

Fig. 2. – Blastema formation and regeneration of the pharynx in the irradiated posterior fragments 3 days after amputation. (A-C), dorsal view of the wound region of the regenerating fragment. (D-F), in situ hybridization of sagittal sections with *Djmhc-A*. Arrowheads indicate regenerating pharynx. (A) and (D), un-irradiated fragment. (B) and (E), fragment with irradiated stump. (C) and (F), completely irradiated fragment.

BMP gene, a key player in dorsoventral patterning, is also expressed in non-regenerating animals (Orii et al., 1998). Thus it is difficult to distinguish between non-regenerating and regenerating animals on the basis of expression of the genes involved in pattern formation.

Wound closure, rather than the blastema, may play an important role in pattern formation. Wound closure and pattern formation occurred even in regenerating fragments with no blastema (Figs. 2B and E). Kato et al. (1999) demonstrated that interactions between dorsal and ventral tissues at the wound could act as a trigger for regeneration and had an important role in blastema formation. Chandebois (1979 & 1980) suggested that contact between the dorsal and ventral epidermis stimulated regeneration, and that the manner of the wound closure played a key role in determining the anteroposterior pattern. It is possible that wound closure acts as an organizer and plays a key role in pattern formation. Based on the results of the present study, I propose the hypothesis that pattern formation occurs in the whole fragment after amputation (Fig. 3). The original pattern is cancelled following amputation and new pattern formation occurs throughout the whole fragment after wound closure. The new pattern may not be formed as the result of an induction sequence, such as that seen during intercalary regeneration between a graft and the surrounding tissue. Thus, the new pattern is not restricted to the stump and blastema. The pluripotent neoblasts then differentiate according to the new pattern. The formation and growth of the blastema is independent of pattern formation.

It has recently been reported that silencing of Wnt signaling by RNA interference for the β-catenin gene resulted in transformation of tail to head characteristics (Gurley et al., 2008; Iglesias et al., 2008; Petersen & Reddien, 2008), while up-regulation of Wnt signaling conversely resulted in transformation of head to tail characteristics (Gurley et al., 2008). These results suggest that Wnt signaling is involved in pattern formation along the anteroposterior axis. However, the temporal and spatial regulation of this signaling mechanism remain to be determined. It is also needed to compare the bases of pattern formation between planarians and other animals with good regeneration abilities, such as hydra and newts.

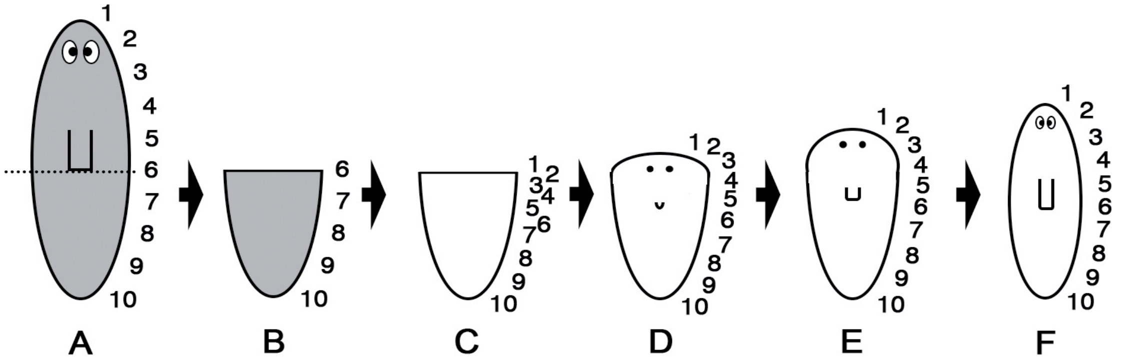

Fig. 3. – A model for pattern formation during regeneration of the posterior fragment. (A) An intact planarian grows according to this pattern. The dotted line indicates the level of amputation. (B) A posterior fragment isolated by amputation. (C) A new pattern is reformed throughout the fragment after wound closure, but before blastema formation. (D) Blastema formation with cell differentiation according to the new pattern. (E) Regeneration including reformation of old tissues proceeds as the blastema grows. (F) Finally, the fragment becomes a small animal with the same shape as the original animal. The pattern is maintained. The regionality of the intact body is indicated by the numbers. 1, 5-6 and 10 indicate the anterior tip, middle part and the posterior tip, respectively.

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Distribution of proliferating cells and *vasa*-positive cells in the embryo of *Macrostomum lignano* (Rhabditophora, Platyhelminthes)

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ABSTRACT. The neoblast stem cell system of flatworms is considered to be unique within the animal kingdom. How this stem cell system arises during embryonic development is intriguing. Therefore we performed bromodeoxyuridine labelling on late stage embryos of *Macrostomum lignano* to assess when the pattern of proliferating cells within the embryo is comparable to that of hatchlings. This pattern can be found in late embryonic stages (stage 8). We also used the freeze cracking method to perform *macvasa* embryonic labelling. *Macvasa* is a somatic and germ line stem cell marker. We showed macvasa protein distribution during the whole embryonic development. In the *macvasa*-positive blastomeres the protein is localized around the nucleus in the putative chromatoid bodies. However, at a specific embryonic stage, it is also ubiquitously present in the cytoplasm of some blastomeres. We compare our data with what is known from Schmidtea polychroa of the expression of the *vasa*-like gene *SpolvlgA* and the protein distribution of the chromatoid body component *Spoltud*-1. The embryonic origin of the somatic stem cell system and the germ line is discussed.

KEY WORDS: Macrostomorpha, embryonic development, neoblasts, markers, germ line.

INTRODUCTION

The neoblast stem cell system of flatworms is considered to be unique within the animal kingdom because it is responsible for the formation of somatic cells as well as cells of the germ line. In all other animals, such totipotency can only be found in the very early embryo. The question thus rises whether neoblasts are embryonic totipotent stem cells that persist in adulthood.

In their study of the embryonic development of *Macrostomum lignano* Ladurner et al., 2005, Morris et al. (2004) were unable to elucidate the intriguing issue of the origin of this unique stem cell system. It could be that it descends from neoblast-like cells found early in embryogenesis (Le Moigne, 1963) or, alternatively, that it arises at a later stage of development or is renewed from differentiated cells (Peter et al., 2004). The distribution of neoblasts is also unclear. Are they located randomly within the embryo or are there specific clusters associated with specific organ primordia?

A substantial number of studies have previously suggested that platyhelminths do not seem to have a separate embryonic germ line, yet it is formed epigenetically during post-embryonic development (references in Zayas et al., 2005). However, Pfister et al. (2008) recently suggested an embryonic segregation of the germ line in *M. lignano,* based on their observation of a cluster of germ line-specific *macvasa*-positive cells (termed gonad anlage) in freshly-hatched worms. To answer the question when exactly the segregation happens, more embryonic data are needed. To resolve all these issues we decided to perform a study to clarify the embryonic nature and origin of neoblasts. We used the freeze cracking method (Willems et al., 2009) to perform *macvasa* embryonic labelling. *Vasa* is highly conserved in all animals (Sato et al., 2006; Wang et al., 2007, see references in Pfister et al., 2008) and seems to be a specific germ cell marker. It is responsible for establishing and maintaining the dichotomy of germ line and soma in animal development (references in Rebscher et al., 2007). Therefore, this marker can be used to elucidate the evolution of germ cell specification (Rebscher et al., 2007).

In *M. lignano*, *vasa* is distributed in male and female gonads and in somatic stem cells in juvenile and adult worms, suggesting that *vasa* also plays a role in neoblast maintenance and differentiation (Pfister et al., 2008). In a broad variety of species, *vasa* has been shown to be present in a perinuclear germ line-specific organelle called nuage (Ikenishi, 1998; Shibata et al., 1999; Knaut et al., 2000; Carre et al., 2002; Findley et al., 2003; Bilinski et al., 2004; Parvinen, 2005; Johnstone et al., 2005) — an evolutionarily conserved structure of unknown function. In flatworms a chromatoid body – a structure similar to nuage – has been found in stem cells (Morita et al., 1969; Coward, 1974; Hori, 1982). Chromatoid bodies disappear during neoblast differentiation but remain in the germ line (Coward, 1974; Hori, 1982; Sato et al., 2001, 2006; Pfister et al., 2008). Recently, Solana & Romero (2009) identified *SpolvlgA*, a *Schmidtea polychroa* homolog of the DDX3/PL10 DEAD-box RNA helicase *DjvlgA* from the planarian species *Dugesia japonica* (Shibata et al., 1999).

SpolvlgA mRNA expression was observed: 1) in blastomeres and embryonic cells in early developmental stages; 2) in embryonic cells during stage 5 of planarian development highlighting massive embryonic cell differentiation and 3) in proliferating and differentiating cells during late developmental stages (Solana & Romero, 2009). Moreover, these authors observed a change in localization of this *vasa*-like gene expression during embryonic development, from perinuclear to cytoplasmic.

In this contribution we show, for the first time, true *vasa* protein distribution in the embryo of *M. lignano* (*macvasa*). We also performed bromodeoxyuridine (BrdU) labelling experiments on embryos during late development to study the distribution of proliferating cells.

MATERIALS AND METHODS

Cultures

Cultures of *M. lignano* were reared in Petri dishes following the protocol of Rieger et al. (1988) and fed with the diatom *Nitzschia curvilineata*. They were maintained in a temperature-controlled chamber at 20°C, 60% humidity, and a photoperiod of 13 h light and 11 h dark.

Staging system

Embryonic stages are named according to the staging system of Morris et al. (2004). The total developmental time (± 120 h at 20 °C) was subdivided in intervals of 15h. Eight stages were assigned to each interval. Stage 3 is characterized by the expansion and diversification of the embryonic primordium. Anteriorly and laterally, cells of smaller size form the primordium of the body wall and nervous system (somatic primordium); large, yolk-rich cells in the centre represent the primordium of the gut (Morris et al., 2004).

BrdU labelling

BrdU is incorporated into the S-phase of the cell cycle. Living embryos at 80% and 95% of their total development time were therefore freed from their egg-shell with electrolytically-sharpened tungsten needles allowing the BrdU to penetrate the embryo during the short (30 min) pulse period. BrdU concentration (5 mM) and protocol were as described by Ladurner et al. (2000).

Vasa labelling

Eggs of all stages were collected and washed (3x) in phosphate-buffered saline (PBS). The egg shell of embryos was permeabilized using the freeze cracking procedure (Willems et al., 2009). Primary antibody and secondary antibody concentrations were 1/200 and 1/150, respectively.

RESULTS AND DISCUSSION

Proliferating cells

Embryos at 80% of their developmental time (stage 7 according to Morris et al., 2004), were the earliest stage at which proliferating cells were found in a specific pattern consisting of two lateral bands (Fig. 1). The number of proliferating cells in embryos at this stage was 57.4 (SD 6.50, n=5). This is in accordance with the neoblast distribution in the juveniles and adults (Rieger et al., 1994 for *Macrostomum hystricinum marinum* ; Ladurner et al., 2000; Bode et al. 2006; Egger et al., 2006 for *M. lignano*) and coincides with the position of the main lateral nerve cords. Remarkably, contrasting with the distribution of proliferating cells in a one-day-old hatchling, we also found BrdU-labelled cells anterior to the eyes. In most embryos, proliferating cells were also found perpendicularly to the longitudinal lateral bands, at the level of the post-pharyngeal commissure. This further corroborates the hypothesis that the nervous system might exert a guiding function on proliferating cells via cell-cell connections or via neurosecretion, as proposed by Baguñà et al. (1989) and Bode et al. (2006), even in the embryo. In embryos at 90% of their total developmental time (stage 8; close to hatching), proliferating cells were found in the same pattern as in one-day-old hatchlings with the exception of a few cells occurring perpendicularly to the lateral bands, at the level of the post-pharyngeal commissure (Fig. 1B). In conclusion, the juvenile pattern of BrdU cells, as observed in hatchlings, can already be found in embryos at 80% of their total developmental time. This suggests that already by this stage, only neoblasts are able to proliferate.

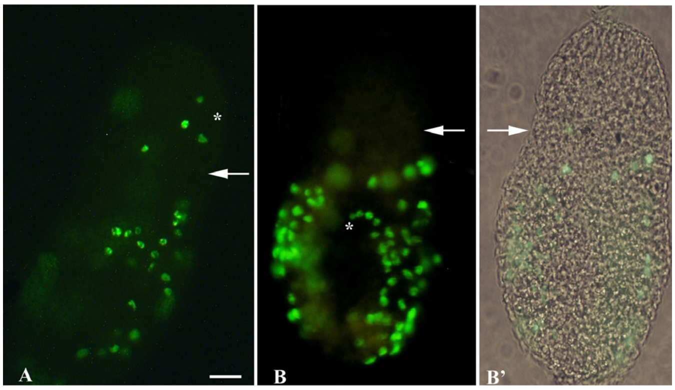

Fig. 1. – Bromodeoxyuridine (BrdU) labelling of stage 7 and stage 8 embryos (80% and 90% of total developmental time). Anterior is to the top. Arrow indicates eye level. Proliferating cells are in green. (A). Stage 7 embryo. Asterisk indicates 3 proliferating cells in front of the eye level. The low number of proliferating cells is due to the organism lying on its side and the epifluorescence image only showing proliferating cells present on the right side. (B). Stage 8 embryo. Asterisk indicates proliferating cells at the position of the post-pharyngeal commissure. (B’) Light microscopical image of the embryo shown in (B). Scale bar for all pictures: 20 µm

Macvasa cells

Macvasa labelling shows different patterns in successive embryonic stages. In the ripe oocyte, stained in adults, the *macvasa* protein is only located in the perinuclear chromatoid bodies (Pfister et al., 2008). The same pattern can be found in a restricted number of cells in stage 1 and 2 embryos (Fig. 2A’-B’). At this stage *macvasa* is located in perinuclear granules in most but not all blastomeres. These granules are probably chromatoid bodies as this localization is extremely similar to subcellular localization of the *Spoltud*-1 protein, a chromatoid body component in *Schmidtea polychroa* (Solana et al., 2009).

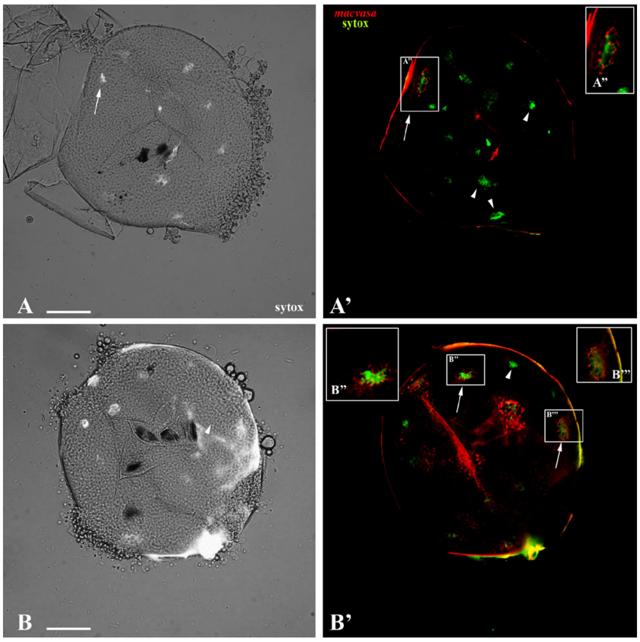

Fig. 2. – *Macvasa* staining of *Macrostomum lignano* stage 1 and 2 embryo (red). Embryos were counterstained with sytox to label nuclei (green). (A) and (B) represents the light microscopical image of the embryo. Sytox is pseudo-coloured white. (A') and (B') show the *macvasa*/sytox labelling as a single confocal stack (one plane) corresponding to the respective embryos depicted in (A) and (B). Insets (A'-A'') and (B''-B''') depict magnification of *macvasa/sytox* positive cells. Arrows point to *macvasa*-positive cells; arrowheads, point to cells that were only labelled with sytox. Note that the *macvasa* protein is perinuclearly expressed in the chromatoid bodies.

In stage 3 embryos, *macvasa* staining is restricted to cells located in an area in the anterior part of the developing embryo (Fig. 3A). The fate of all other cells has probably already been determined. In some of the *macvasa*-positive cells only chromatoid bodies are stained, but in the majority of these cells the entire cytoplasm is stained and single chromatoid bodies cannot be discerned (inset of Fig 3A'-A''). These *macvasa*-positive cells are the cell pool that will form the neoblast system and the gonads. This immediately poses the intriguing question: why, in stage 3 embryos, do the majority of *macvasa*-positive cells have the complete cytoplasm stained instead of only the chromatoid bodies? Solana & Romero (2009) found a similar cytoplasmic distribution for the gene *SpolvlgA* during stage 5 embryos of *Schmidtea polychroa*. Two possible reasons can be conjectured: 1) *vasa* is generally seen as essential for germ line development but it could also have a broader function in stem cells. The *vasa*-positive cells in the early embryo would then represent embryonic stem cells. *Vasa* up-regulation would function as a restrictor, preventing these cells from differentiating during early embryonic development, when most other tissues and organs start to differentiate. In this way, a population of embryonic stem cells is set aside. These cells would later in development give rise to a separate germ line (see further). 2) *macvasa* could have a totally different function during early development. A similar situation is observed with *nanos* expression (a gene similar to *vasa*) in the acoel *Isodiametra pulchra*. *Nanos* is a germ-line marker but also a dorsal determinant during early embryogenesis (De Mulder, pers. comm.).

After stage 3, the *macvasa* protein could only be found in the perinuclear chromatoid bodies (Fig. 3B). From stages 6-8 we observed a separate cluster of *macvasa*-positive cells that showed strong staining intensity in comparison to the other cells (Fig. C-D). Probably, these cell clusters correspond to the gonad anlage found by Pfister et al. (2008) in one-hour hatchlings. Here up-regulation of *vasa* during stages

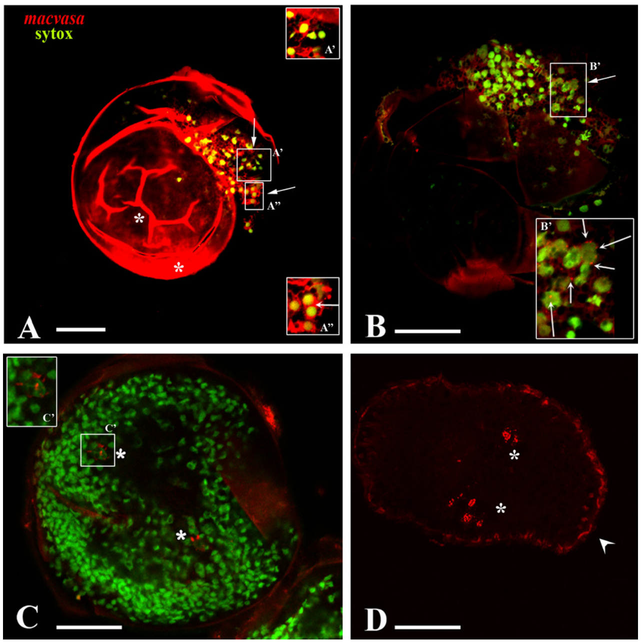

Fig. 3. – *Macvasa* staining of *Macrostomum lignano* stage 3 (A), stage 5 (B), stage 6 (C) and stage 8 (D) embryos (red). Embryos were counterstained with sytox to label nuclei (green). (A) represents a confocal image (one plane) of a stage 3 embryo. Anterior is to the top. Insets (A'-A'') are magnifications of labelled cells. Arrows indicate the *macvasa*-positive cells where the *macvasa* protein is ubiquitously localised in the cytoplasm. The strong background of the eggshell should not be taken into account because the secondary antibody tends to stick to the outside of the eggshell. (B) confocal image of a stage 5 embryo (one plane). The inset (B') shows a magnified detail of the *macvasa* positive cells (arrow). Note the localization of *macvasa* in the perinuclear granules or chromatoid bodies (B', arrows). (C) Stage 6 embryo. *Macvasa*-positive cells are, for the first time, located bilaterally in the embryo. A limited number of cells, both on the left and right side of the embryo, show strong *macvasa*-positive signal (asterisks). Note that if we would over saturate the image, other *macvasa*-positive cells would become apparent (not shown). (D). Stage 8 embryo (only *macvasa*). The embryo has already a worm like shape (posterior indicated by arrowhead). Only a cluster of cells shows strong *macvasa* signal in chromatoid bodies (asterisks). The fact that no somatic *macvasa*-positive neoblasts are visible can be attributed to the weaker signal of these cells in comparison to the cluster of cells corresponding to the putative germ line precursors. This low signal may not have been significantly detected by our protocol, although we used the same primary and secondary antibody concentrations as in Pfister et al., 2008). Scale bar: 20 µm, except D: 50 µm

6-8 could represent a restriction mechanism to prevent the precursors of the germ line from differentiating into somatic tissues (Fig. 3 C-D). Possibly, the cell cluster corresponds to a separated germ line during late embryogenesis. These results suggest that by stages 6- 8 of embryogenesis, the specification of the germ line has already taken place and primordial germ cells are in their normal position, where the mature gonad will develop. Recent data on *Smednos* in *Schmidtea meditteranea* stage 8 embryos (Handberg-Thorsager & Salo, 2007) indicate a similar mechanism. Our results of *macvasa* expression during *Macrostomum* embryogenesis provide new evidence that strengthens the hypothesis of Pfister et al. (2008), who stated that the germ line in *Macrostomum* is segregated embryonically.

One should be very cautious when comparing gene expression data with protein localization. Yet, the changes in subcellular localization of *macvasa* bear some similarities with the expression pattern of the *SpolvlgA* gene during the embryonic development of *Schmidtea polychroa* and with the localization of the *Spoltud-1* protein (Solana & Romero, 2009; Solana et al., 2009). Distribution of the *macvasa* protein changes during early development from being in chromatoid bodies to a cytoplasmic distribution pattern, coinciding with waves of cellular differentiation later on, and finally again to a location in chromatoid bodies. However, there are also some notable differences: 1) distribution in the early embryo was restricted to some but not all blastomeres; 2) a specific distribution pattern of the *macvasa*-positive cells was observed only in the anterior of the embryo at stage 3 of development, and finally 3) a stronger label was observed in two clusters of cells in comparison to the remaining embryonic neoblasts, after stage 6. These clusters could be the precursor of the germ line.

In conclusion we identified, for the first time, the distribution of a true *vasa* protein in blastomeres of a flatworm embryo. Unfortunately, *macvasa* labels both germ line and neoblast, which makes it very difficult to discern both cell lines. In the future this type of study awaits a true neoblast or germ-line marker before any final conclusions on the germ line specification mechanism in *M. lignano* can be drawn.

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Parasites of coral reef fish: how much do we know? With a bibliography of fish parasites in New Caledonia

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ABSTRACT. A compilation of 107 references dealing with fish parasites in New Caledonia permitted the production of a parasite-host list and a host-parasite list. The lists include Turbellaria, Monopisthocotylea, Polyopisthocotylea, Digenea, Cestoda, Nematoda, Copepoda, Isopoda, Acanthocephala and Hirudinea, with 580 host-parasite combinations, corresponding with more than 370 species of parasites. Protozoa are not included. Platyhelminthes are the major group, with 239 species, including 98 monopisthocotylean monogeneans and 105 digeneans. Copepods include 61 records, and nematodes include 41 records. The list of fish recorded with parasites includes 195 species, in which most (ca. 170 species) are coral reef associated, the rest being a few deep-sea, pelagic or freshwater fishes. The serranids, lethrinids and lutjanids are the most commonly represented fish families. Although a list of published records does not provide a reliable estimate of biodiversity because of the important bias in publications being mainly in the domain of interest of the authors, it provides a basis to compare parasite biodiversity with other localities, and especially with other coral reefs. The present list is probably the most complete published account of parasite biodiversity of coral reef fishes. However, it is estimated that the present state of knowledge (370 parasite species) represents only 2% of the possible number of metazoan parasites of fish present in a coral reef environment.

KEY WORDS: Check lists, parasite-hostlist, host-parasit list, biodiversity

RÉSUMÉ: Une compilation de 107 références traitant de parasites de poissons en Nouvelle-Calédonie a permis de produire une liste parasites-hôtes et une liste hôtes-parasites. Les listes incluent des Turbellaria, Monopisthocotylea, Polyopisthocotylea, Digenea, Cestoda, Nematoda, Copepoda, Isopoda, Acanthocephala et Hirudinea, avec 580 combinaisons hôtes-parasites, correspondant à plus de 370 espèces de parasites. Les Protozoa ne sont pas inclus. Les Plathelminthes sont le groupe le plus important avec 239 espèces, y compris 98 monogènes Monopisthocotylea et 105 digènes. Les copépodes incluent 61 mentions, et les nématodes 41. La liste des poissons mentionnés avec des parasites inclut 195 espèces, parmi lesquels la plupart (environ 170 espèces) sont associées aux récifs coralliens, le reste étant quelques espèces de mer profonde, pélagiques ou d'eau douce. Les Serranidae, Lethrinidae et Lutjanidae sont les familles les plus représentées. Bien qu'une liste de mentions publiées ne fournisse pas une estimation fiable de la biodiversité à cause du biais important des publications, qui sont produites principalement dans les domaines d'intérêt des auteurs, elle forme une base pour comparer la biodiversité parasitaire avec d'autres localités, et surtout avec d'autres récifs coralliens. Cette liste est probablement la plus complète publiée pour la biodiversité parasitaire des poissons des récifs coralliens. Toutefois, on estime que l'état actuel de nos connaissances (370 espèces de parasites) ne représente que 2% du nombre possible de parasites métazoaires de poissons présents dans l'environnement des récifs coralliens.

INTRODUCTION

Coral reefs are areas of great biodiversity (Reaka-Kudla, 1997). The lagoon around the mainland of New Caledonia is the largest coral lagoon in the World and its fauna is probably one of the best known, with 8,299 metazoan species recorded and identified, including 1,694 in-shore fishes (Fricke & Kulbicki, 2007). Thus, New Caledonia is a good example of coral reef biodiversity and results obtained at this location provide a basis for comparison with other coral reef environments.

Parasites generally represent a neglected compartment of diversity, because they are small, hidden on or within their hosts, and need more detailed observation and preparation than vertebrates and large invertebrates to be identified with precision.

In this study, I attempt to evaluate what is currently known about the fauna of fish parasites in New Caledonia, and to estimate the extent of our current knowledge in comparison to a total, exhaustive (and probably impossible to reach) inventory of the parasite fauna.

MATERIALS AND METHODS

The present evaluation is based only on published records. Although every effort has been made to compile a complete list, it is still possible that a few references have been overlooked. Among the studies of fish parasites in

New Caledonia, a major work was the study on digeneans by Manter, who came for three weeks in 1963 and then described more than 40 species (Durio & Manter, 1968a, b, 1969; Manter, 1969). Unfortunately, many fishes from which Manter collected digeneans were not identified to the species level. The vernacular New Caledonian names used by Manter have been 'translated' into binomials (Justine, 2007a). A number of papers by various authors on various groups and mainly based on the Australian fauna, include records from New Caledonia. The present author began a detailed study of fish parasites in 2003 and collected specimens from all groups; most recent papers have benefited from these collections. Host names have been updated using FishBase (Froese & Pauly, 2009). Papers with insufficient levels of taxonomic identification (Morand et al., 2000; Sasal et al., 2007) are not included within the lists.

Table 1 includes a parasite-host list, in which all parasites recorded are listed in taxonomical order (and alphabetical order within each group) and their fish host is indicated.

Monogeneans have been separated as Monopisthocotylea and Polyopisthocotylea, because these two groups are generally very different in size and are easily distinguished at the time of collection, and also because doubts about the monophyly of monogeneans (Justine, 1998; Park et al., 2007) suggest that these two groups should be treated independently. Monogenean genera (*Haliotrema*, etc.) sometimes included in the Dactylogyridae are here considered as members of the Ancyrocephalidae. Cestodes have been separated as three subgroups, or orders (Tetraphyllidea, Bothriocephalidea and Trypanorhyncha) because these classes represent very different forms and are not collected using the same methods (larval trypanorhynchs are generally encysted and require special manipulation); pseudophyllideans are classified as Bothriocephalidea (Kuchta et al., 2008).

Species counts generally include unidentified species, but these are counted only once. This certainly minimizes the number of species, since it is likely, for example, that most *Haliotrema* spp. or *Euryhaliotrema* spp. (monogeneans) represent different species.

Table 2 is a host-parasite list, in which each fish has its parasites listed. Elasmobranch and teleost fish are separated, and fish families are listed in alphabetical order, as are species within each family. When a parasite species has been recorded under several different names, it is designated "as other name" in the lists, to avoid its record being counted twice. Absence of known hosts in the case of certain isopods comes from the fact that some of these parasites were collected in dredges.

RESULTS

A total of 109 references include records of fish parasites in New Caledonia. These represent more than 1,500 published pages (excluding books).

Table 1 (the parasite-host list) includes 371 species of parasites (unidentified species have been counted as one species, thus minimizing the count). The total number of host-parasite combinations is 580. Table 3 summarizes the numerical importance of each parasitic group. Platyhelminthes is the major group with 240 species, representing 65% of the total number of species, followed by copepods (61 records, 16%) and nematodes (41 records, 11%). Monopisthocotylean monogeneans, with 98 species, include only 4 families (Ancyrocephalidae, Capsalidae, Diplectanidae and Monocotylidae) but diplectanids alone include 58 species. Digeneans include 20 families and 105 species; the most speciose families are the Opecoelidae (18 species) and Hemiuridae (17). Nematodes include 9 families; the most speciose families are the Camallanidae (10 species) and Philometridae (9).

Table 2 (the host-parasite list) includes 195 fish species with parasites recorded. This includes 14 elasmobranch and 181 teleost species. The families with the most species mentioned with parasites are the serranids (30), lethrinids (16), lutjanids (15) and chaetodontids (15). The fish species with the highest numbers of published parasite species are the balistid *Pseudobalistes fuscus* (12 species), the lethrinid *Lethrinus nebulosus* (13), and the serranids *Epinephelus fasciatus* (15), *E. maculatus* (16), and *E. cyanopodus* (31). Exhaustive counts of parasite species have been attempted previously for a few species of serranids (Table 4). Although impressive in the number of species listed, these lists are not complete; it has been suggested that about 100 species of parasites might be present in the large species of groupers (serranids) (Justine & Sigura, 2007). However, these estimates are of interest in showing that the present list of published records includes only a small proportion of the actual parasite biodiversity.

Most fish recorded here are from inside the lagoon and thus should be considered as coral reef-associated species. A few fish (4) mentioned in the list have been collected in freshwater and are indicated as such. However, most freshwater species on Pacific islands have at least a part of their life-cycle in sea and thus it is not aberrant to include them in the list. A few species (16) are deep-sea fishes and are indicated as such; they harbour less than 40 parasite species. The serranid *Epinephelus chlorostigma* is mentioned as a deep-sea fish because the specimen was actually caught on the outer slope of the barrier reef, but may be encountered in shallow waters (Fricke & Kulbicki, 2007). In contrast, there is no doubt that the deep-sea sharks, the lutjanids *Etelis* spp. and *Pristipomoides* spp. and the hoplichthyid *Hoplichthys citrinus* are strictly confined to deep-sea environments. A few species (2-4) are pelagic fish, such as the large scombrids (tunas and swordfish). Finally, of the 195 fish species recorded with parasites, more than 170 can be considered as coral-associated lagoon fish; parasite species recorded from coral reef fish are about 350.

DISCUSSION

A faunal list based on published references certainly does not show the actual importance of biodiversity. A main reason is that authors publish on the groups that are of interest to them, for personal or scientific reasons. As an example, the high numbers of diplectanid monogeneans or trichosomoidid nematodes recorded are simply an indication of the interest of the present author in these groups, and does not indicate that the parasite fauna of New Caledonia is specially rich in these families. Another reason is that trivial groups, which are known 'everywhere' and considered as of little interest, are often simply not mentioned; this is probably the case for anisakid nematode larvae, which are present in nearly all fish examined, but rarely mentioned. Tetraphyllidean cestode larvae or gnathiid isopod larvae are also good example of widespread biodiversity, generally neglected in publications. For certain groups, we already know that biodiversity (the actual number of species) is much higher than the one already described (e.g. see Table 2 in Kritsky et al., 2009) but taxonomical work is still in progress and unpublished.

However, a list based on references shows the extent of knowledge which is actually available to the scientific community, and allows comparisons with other geographical localities, i.e. we can compare parasite biodiversity in New Caledonia with other localities only on the basis of published works. Table 5 summarizes available references on fish parasites in the Indo-Pacific. Most studies listed here do not allow comparison of the relative parasite biodiversity in coral reefs, because they include fishes from freshwater or from noncoral associated environments. The only list which was specifically limited to a coral-reef environment, Heron Island on the Great Barrier Reef (Lester & Sewell, 1989), was the result of both a one-week meeting and field work by a group of parasitologists in 1986 and a compilation of the available literature at that period. This list included only 122 fish species (compared to about 170 coral-reef fishes in the present compilation); the number of records (i.e. host-parasite combinations) is the same in both studies (580) but this includes all parasites, including Protozoa, on Heron Island, compared with only Metazoa in New Caledonia. The number of parasite species on Heron Island was not indicated, and many identifications were at the family level only; it can be estimated to be about 300, in comparison to 370 in New Caledonia. Therefore, the present study is likely to be the most complete compilation of metazoan parasites of coral reef fishes in the literature.

An evaluation of global parasite biodiversity in coral reef fishes

orders

Evaluations of numbers of parasites per fish species have been proposed by various authors (Table 5). The early global estimate of Rohde of 20,000 parasite species (including Protozoa) for 1,000 fish species on Heron Island is generally considered reasonable by most authors. A prediction of 100,000–200,000 helminth species for the 20,000 species of fish worldwide has also been made (Bouchet, 2006).

The number of fish species in New Caledonia is about 2,200. Precise counts include 1,694 "in-shore fishes", i.e. fishes encountered at less than 100 m depth (Fricke & Kulbicki, 2007), which we may generally consider as "coral reef associated" and 414 bathyal fishes (Richer de Forges & Justine, 2006); and pelagic species should be added to these partial totals. The fish species recorded with at least one parasite represent about 9% (195: 2,200) of the total number of fish.

The author's present work in New Caledonia (about 1,800 host-parasite combinations, largely unpublished) suggests that an average of 10 species of parasites per fish species is a reasonable estimate (only Metazoa, excluding Protozoa). Large species might harbour as many as 100 species of parasites (Justine & Sigura, 2007). This allows a prediction of about 17,000 parasites species to be made for the 1,700 reef fishes of New Caledonia. With 350 species recorded, the present bibliographical list highlights the paucity of our knowledge: just 2% of the parasites have been recorded.

Finding 2% of the total number of parasites on 9% of the total number of fish does not suggest that the fish investigated had specially few parasites; this rather shows that most published records were incidental and not the product of an exhaustive survey.

In addition to fish parasite, coral reef include a high number of other parasites or symbionts, such as copepods associated with echinoderms or corals (Boxshall & Huys, 2007), or parasitic molluscs (Bouchet, 2006; Bouchet et al., 2002); the present compilation probably represent a small part of parasite biodiversity in coral reefs.

Many comments have been done about the 'taxonomic impediment' i.e. the difficulty to describe biodiversity. I will just add yet another estimate: given that these 2% needed 1,500 published pages, the publication of parasite biodiversity of New Caledonia would need about 75,000 pages. Taxonomists, who know what amount of work is sometimes hidden behind a single figure or a sentence in a diagnosis, will understand.

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Many authors cited here provided literature. Geoff Boxshall and Jean-Paul Trilles edited the copepods and isopods, respectively. Although the journal recommends that all authors of taxa should be cited, the Editor agreed that the reference list does not include the authors of fish taxa (which are cited without year as it is usual in the parasitological literature), and that authors of parasite taxa are cited only after 1950; in spite of this, authors of taxa represent more than 80 references. Ian Beveridge edited the English.

This compilation includes all publications known to the auther (including in press) in November 2009.

TABLE 1.

Parasite-Host list

Turbellaria

1 family recorded; 1 species recorded + unidentified species

Genostomatidae

***Piscinquilinus* sp.**

Naso unicornis	Justine et al., 2009b

Unknown Family

Acanthurus xanthopterus	Justine et al., 2009b
Chaetodon ephippium	Justine et al., 2009b
Cheilinus chlorourus	Justine et al., 2009b
Chromis viridis	Justine et al., 2009b
Epinephelus fasciatus	Justine et al., 2009b
Nemipterus furcosus	Justine et al., 2009b
Thalassoma lutescens	Justine et al., 2009b

Monogenea Monopisthocotylea

alphabetical order of families and species;
4 families recorded; 98 species recorded + unidentified species

Ancyrocephalidae

***Aliatrema cribbi* Plaisance & Kritsky, 2004**

Chaetodon auriga	Plaisance & Kritsky, 2004
Chaetodon vagabundus	Plaisance & Kritsky, 2004
Heniochus chrysostomus	Plaisance & Kritsky, 2004

***Euryhaliotrematoides annulicirrus* (Yamaguti, 1968) Plaisance & Kritsky, 2004**

Chaetodon auriga	Plaisance & Kritsky, 2004
Chaetodon vagabundus	Plaisance & Kritsky, 2004

***Euryhaliotrematoides aspitis* Plaisance & Kritsky, 2004**

Chaetodon vagabundus	Plaisance & Kritsky, 2004

***Euryhaliotrematoides grandis* (Mizelle & Kritsky, 1969) Plaisance & Kritsky, 2004**

Chaetodon auriga	Plaisance & Kritsky, 2004
Chaetodon vagabundus	Plaisance & Kritsky, 2004

***Euryhaliotrematoides microphallus* (Yamaguti, 1968) Plaisance & Kritsky, 2004**

Heniochus chrysostomus	Plaisance & Kritsky, 2004

***Euryhaliotrematoides pirulum* Plaisance & Kritsky, 2004**

Chaetodon auriga	Plaisance & Kritsky, 2004

***Haliotrema aurigae* (Yamaguti, 1968) Plaisance, Bouamer & Morand, 2004**

Chaetodon auriga	Plaisance et al., 2004
Chaetodon citrinellus	Plaisance et al., 2004
Chaetodon vagabundus	Plaisance et al., 2004
Heniochus chrysostomus	Plaisance et al., 2004

***Haliotrema banana* Lim & Justine, 2007**

Bodianus perditio	Lim & Justine, 2007

***Haliotrema epinepheli* Young, 1968**

Epinephelus maculatus	Justine, 2007c
Variola albimarginata	Justine, 2005c
Variola louti	Justine, 2005c

***Haliotrema* spp.**

Cephalopholis sonnerati	Justine, 2007d
Epinephelus cyanopodus	Sigura & Justine, 2008
Epinephelus maculatus	Justine, 2007c
Epinephelus morrhua	Justine, 2008c
Lethrinus harak	Justine, 2007e; Rascalou & Justine, 2007
Lethrinus lentjan	Justine, 2007e
Lethrinus nebulosus	Justine, 2007e; Rascalou & Justine, 2007
Lethrinus ravus	Justine, 2007e
Lethrinus rubrioperculatus	Justine, 2007e
Lethrinus xanthochilus	Justine, 2007e

***Euryhaliotrema* spp.**

Lutjanus argentimaculatus	Kritsky et al., 2009
Lutjanus fulviflamma	Kritsky et al., 2009
Lutjanus fulvus	Kritsky et al., 2009
Lutjanus quinquelineatus	Kritsky et al., 2009
Lutjanus russellii	Kritsky et al., 2009
Lutjanus vitta	Kritsky et al., 2009

***Haliotrematoides longitubocirrus* (Bychowsky & Nagibina, 1971) Kritsky, Yang & Sun, 2009**

Lutjanus fulvus	Kritsky et al., 2009
Lutjanus quinquelineatus	Kritsky et al., 2009
Lutjanus russellii	Kritsky et al., 2009
Lutjanus vitta	Kritsky et al., 2009

***Haliotrematoides patellacirrus* (Bychowsky & Nagibina, 1971) Kritsky, Yang & Sun, 2009**

Lutjanus fulviflamma	Kritsky et al., 2009
Lutjanus fulvus	Kritsky et al., 2009
Lutjanus quinquelineatus	Kritsky et al., 2009
Lutjanus russellii	Kritsky et al., 2009
Lutjanus vitta	Kritsky et al., 2009

***Haliotrematoides tainophallus* Kritsky & Justine, 2009 in Kritsky, Yang & Sun**

Lutjanus fulviflamma	Kritsky et al., 2009

***Haliotrematoides potens* Kritsky & Justine, 2009 in Kritsky, Yang & Sun**

Lutjanus argentimaculatus	Kritsky et al., 2009

***Haliotrematoides novaecaledoniae* Kritsky & Justine, 2009 in Kritsky, Yang & Sun**

Lutjanus argentimaculatus	Kritsky et al., 2009

***Haliotrematoides lanx* Kritsky & Justine, 2009 in Kritsky, Yang & Sun**

Lutjanus quinquelineatus	Kritsky et al., 2009

***Pennulituba cymansis* Řehulková, Justine & Gelnar, 2009**

Mulloidichthys vanicolensis	Řehulková et al., 2009

***Pennulituba piratifalx* Řehulková, Justine & Gelnar, 2009**

Mulloidichthys vanicolensis	Řehulková et al., 2009

Tetrancistrum sigani **Goto & Kikuchi, 1917**	
as ***Tetrancistrum nebulosi*** **Young, 1967**	
(synonymy: Kritsky et al., 2007)	
Siganus canaliculatus	Young, 1967a
Siganus fuscescens	Young, 1967a
Siganus sp.	Young, 1967a
Volsellituba elephantina **Řehulková, Justine & Gelnar, 2009**	
Mulloidichthys vanicolensis	Řehulková et al., 2009
Volsellituba nabla **Řehulková, Justine & Gelnar, 2009**	
Mulloidichthys vanicolensis	Řehulková et al., 2009
Volsellituba orchidea **Řehulková, Justine & Gelnar, 2009**	
Mulloidichthys vanicolensis	Řehulková et al., 2009
Unidentified species	
Lutjanus fulvus	Kritsky et al., 2009
Lutjanus kasmira	Kritsky et al., 2009
Lutjanus quinquelineatus	Kritsky et al., 2009
Lutjanus vitta	Kritsky et al., 2009
CAPSALIDAE	
Allobenedenia **sp.**	
Epinephelus cyanopodus	Sigura & Justine, 2008
Benedenia **cf.** ***epinepheli*** **(Yamaguti, 1937)**	
Epinephelus fasciatus	Justine, 2005b
Benedenia **sp.**	
Epinephelus merra	Hinsinger & Justine, 2006a
Nemipterus furcosus	Justine & Brena, 2009
Encotyllabe **sp.**	
Lethrinus harak	Rascalou & Justine, 2007
Lethrinus nebulosus	Rascalou & Justine, 2007
Lethrinus rubrioperculatus	Justine, 2007e
Lethrinus xanthochilus	Justine, 2007e
Dioncopseudobenedenia kala **Yamaguti, 1965**	
Naso unicornis	Perkins et al., 2009
Pseudonitzschia uku **Yamaguti, 1965**	
Aprion virescens	Perkins et al., 2009
Trochopodinae Gen. sp. 1	
Pseudobalistes fuscus	Perkins et al., 2009
Trochopodinae Gen. sp.	
Plectropomus leopardus	Justine & Euzet, 2006
as Trochopodinae Gen. sp. 2	
Plectropomus leopardus	Perkins et al., 2009
Trochopodinae Gen. sp. 4	
Epinephelus fuscoguttatus	Perkins et al., 2009
Capsalidae, unidentified immature	
Sphyraena sp. (recruiting larva)	Cribb et al., 2000
Capsalidae sp. 1	
Plectorhinchus chaetodonoides	Perkins et al., 2009

DIPLECTANIDAE	
Calydiscoides australis **Young, 1969**	
Lethrinus miniatus	Justine, 2007e
Calydiscoides difficilis **(Yamaguti, 1953) Young, 1969**	
Lethrinus harak	Rascalou & Justine, 2007
Lethrinus lentjan	Rascalou & Justine, 2007
Lethrinus nebulosus	Rascalou & Justine, 2007
Lethrinus obsoletus	Rascalou & Justine, 2007
Lethrinus ravus	Rascalou & Justine, 2007
Calydiscoides duplicostatus **(Yamaguti, 1953) Young, 1969**	
Lethrinus harak	Rascalou & Justine, 2007
Lethrinus lentjan	Rascalou & Justine, 2007
Lethrinus nebulosus	Rascalou & Justine, 2007
Calydiscoides gussevi **Oliver, 1984**	
Lethrinus miniatus	Justine, 2007e
Calydiscoides euzeti **Justine, 2007**	
Lethrinus rubrioperculatus	Justine, 2007e
Lethrinus xanthochilus	Justine, 2007e
Calydiscoides limae **Justine & Brena, 2009**	
Pentapodus aureofasciatus	Justine & Brena, 2009
Calydiscoides rohdei **Oliver, 1984**	
Lethrinus atkinsoni	Justine 2007e
Calydiscoides terpsichore **Rascalou & Justine, 2007**	
Lethrinus harak	Rascalou & Justine, 2007
Lethrinus nebulosus	Rascalou & Justine, 2007
Diplectanum uitoe **Justine, 2007**	
Epinephelus maculatus	Justine, 2007c
Diplectanum maa **Justine & Sigura, 2007**	
Epinephelus malabaricus	Justine & Sigura, 2007
Diplectanum nanus **Justine, 2007**	
Cephalopholis sonnerati	Justine, 2007d
Diplectanum parvus **Justine, 2008**	
Cephalopholis urodeta	Justine, 2008a
Echinoplectanum chauvetorum **Justine & Euzet, 2006**	
Plectropomus laevis	Justine & Euzet, 2006
Echinoplectanum laeve **Justine & Euzet, 2006**	
Plectropomus laevis	Justine & Euzet, 2006
Echinoplectanum leopardi **Justine & Euzet, 2006**	
Plectropomus leopardus	Justine & Euzet, 2006
Echinoplectanum pudicum **Justine & Euzet, 2006**	
Plectropomus leopardus	Justine & Euzet, 2006
Echinoplectanum rarum **Justine & Euzet, 2006**	
Plectropomus leopardus	Justine & Euzet, 2006
Lamellodiscus **sp.**	
Gnathodentex aureolineatus	Justine, 2007e; Justine & Briand, 2009

Lamellodiscus magnicornis **Justine & Briand, 2009**

Gymnocranius euanus	Justine & Briand, 2009
Gymnocranius grandoculis	Justine & Briand, 2009
Gymnocranius sp. A	Justine & Briand, 2009
Gymnocranius sp. B	Justine & Briand, 2009
as *Lamellodiscus* sp.	
Gymnocranius euanus	Justine, 2007e
Gymnocranius grandoculis	Justine, 2007e

Lamellodiscus parvicornis **Justine & Briand, 2009**

Gymnocranius euanus	Justine & Briand, 2009
Gymnocranius grandoculis	Justine & Briand, 2009
Gymnocranius sp. B	Justine & Briand, 2009
as *Lamellodiscus* sp.	
Gymnocranius euanus	Justine, 2007e
Gymnocranius grandoculis	Justine, 2007e

Lamellodiscus tubulicornis **Justine & Briand, 2009**

Gymnocranius grandoculis	Justine & Briand, 2009
Gymnocranius sp. B	Justine & Briand, 2009
as *Lamellodiscus* sp.	
Gymnocranius grandoculis	Justine, 2007e

Laticola cyanus **Sigura & Justine, 2008**

Epinephelus cyanopodus	Sigura & Justine, 2008

Laticola dae **Journo & Justine, 2006**

Epinephelus maculatus	Journo & Justine, 2006

Pseudorhabdosynochus auitoe **Justine, 2007**

Epinephelus maculatus	Justine, 2007c

Pseudorhabdosynochus argus **Justine, 2007**

Cephalopholis argus	Justine, 2007d

Pseudorhabdosynochus bacchus **Sigura, Chauvet & Justine, 2007**

Epinephelus coeruleopunctatus	Sigura et al., 2007

Pseudorhabdosynochus buitoe **Justine, 2007**

Epinephelus maculatus	Justine, 2007c

Pseudorhabdosynochus calathus **Hinsinger & Justine, 2006**

Epinephelus rivulatus	Hinsinger & Justine, 2006a

Pseudorhabdosynochus caledonicus **Justine, 2005**

Epinephelus fasciatus	Hinsinger & Justine, 2006a; Justine, 2005b

Pseudorhabdosynochus chauveti **Sigura & Justine, 2008**

Epinephelus cyanopodus	Sigura & Justine, 2008

Pseudorhabdosynochus cuitoe **Justine, 2007**

Epinephelus maculatus	Justine, 2007c

Pseudorhabdosynochus cyanopodus **Sigura & Justine, 2008**

Epinephelus cyanopodus	Sigura & Justine, 2008

Pseudorhabdosynochus cyathus **Hinsinger & Justine, 2006**

Epinephelus howlandi	Hinsinger & Justine, 2006a

Pseudorhabdosynochus duitoe **Justine, 2007**

Epinephelus maculatus	Justine, 2007c
Epinephelus cyanopodus	Sigura & Justine, 2008

Pseudorhabdosynochus epinepheli **(Yamaguti, 1938) Kritsky & Beverley-Burton, 1986**

Epinephelus chlorostigma	Justine, 2009b

Pseudorhabdosynochus euitoe **Justine, 2006**

Epinephelus maculatus	Justine, 2007c

Pseudorhabdosynochus exoticus **Sigura & Justine, 2008**

Epinephelus cyanopodus	Sigura & Justine, 2008

Pseudorhabdosynochus fuitoe **Justine, 2007**

Epinephelus maculatus	Justine, 2007c

Pseudorhabdosynochus guitoe **Justine, 2007**

Epinephelus maculatus	Justine, 2007c

Pseudorhabdosynochus hirundineus **Justine, 2005**

Variola louti	Justine, 2005c

Pseudorhabdosynochus inversus **Justine, 2008**

Epinephelus rivulatus	Justine, 2008b

Pseudorhabdosynochus huitoe **Justine, 2007**

Epinephelus maculatus	Justine, 2007c
Epinephelus cyanopodus	Sigura & Justine, 2008

Pseudorhabdosynochus manifestus **Justine & Sigura, 2007**

Epinephelus malabaricus	Justine & Sigura, 2007

Pseudorhabdosynochus malabaricus **Justine & Sigura, 2007**

Epinephelus malabaricus	Justine & Sigura, 2007

Pseudorhabdosynochus maternus **Justine & Sigura, 2007**

Epinephelus malabaricus	Justine & Sigura, 2007

Pseudorhabdosynochus manipulus **Justine & Sigura, 2007**

Epinephelus malabaricus	Justine & Sigura, 2007

Pseudorhabdosynochus marcellus **Justine & Sigura, 2007**

Epinephelus malabaricus	Justine & Sigura, 2007

Pseudorhabdosynochus maaensis **Justine & Sigura, 2007**

Epinephelus malabaricus	Justine & Sigura, 2007

Pseudorhabdosynochus melanesiensis **(Laird, 1958) Kritsky & Beverley-Burton, 1986**

Epinephelus merra	Hinsinger & Justine, 2006a; Justine, 2005b

Pseudorhabdosynochus minutus **Justine, 2007**

Cephalopholis sonnerati	Justine, 2007d

Pseudorhabdosynochus morrhua **Justine, 2008**

Epinephelus morrhua	Justine, 2008c

Pseudorhabdosynochus podocyanus **Sigura & Justine, 2008**

Epinephelus cyanopodus	Sigura & Justine, 2008

***Pseudorhabdosynochus* cf. *shenzhenensis* Yang, Zeng & Gibson, 2005**
Epinephelus malabaricus Justine & Sigura, 2007

***Pseudorhabdosynochus variabilis* Justine, 2008**
Epinephelus morrhua Justine, 2008c

***Pseudorhabdosynochus venus* Hinsinger & Justine, 2006**
Epinephelus howlandi Hinsinger & Justine, 2006b

***Pseudorhabdosynochus youngi* Justine, Dupoux & Cribb, 2009**
Epinephelus fasciatus Justine et al., 2009a
as *Ps. cupatus* (Young, 1969) Kritsky & Beverley-Burton, 1986
Epinephelus fasciatus Hinsinger & Justine, 2006a; Justine, 2005b

***Pseudorhabdosynochus* cf. *coioidesis* Bu, Leong, Wong , Woo & Foo, 1999**
Epinephelus merra Hinsinger & Justine, 2006a

***Pseudorhabdosynochus* spp.**
Epinephelus coeruleopunctatus Sigura et al., 2007

Monocotylidae

***Decacotyle octona* (Young, 1967) Chisholm & Whittington, 1998**
Aetobatus cf. *narinari* Marie & Justine, 2005

***Decacotyle elpora* Marie & Justine, 2005**
Aetobatus cf. *narinari* Marie & Justine, 2005

***Clemacotyle australis* Young, 1967**
Aetobatus cf. *narinari* Marie & Justine, 2005

***Thaumatocotyle pseudodasybatis* Hargis, 1955**
Aetobatus cf. *narinari* Marie & Justine, 2005, 2006

***Triloculotrema chisholmae* Justine, 2009**
Squalus melanurus Justine, 2009a

Monogenea polyopisthocotylea

(alphabetical order of families and species;
5 families recorded; 8 species recorded + unidentified species)

Axinidae

***Allopseudaxine* sp.**
Katsuwonus pelamis Rohde et al., 1980

***Allopseudaxinoides vagans* (Ishii, 1936) Yamaguti, 1968**
Katsuwonus pelamis Rohde et al., 1980

Gastrocotylidae

***Neothoracocotyle acanthocybii* (Meserve, 1938) Hargis, 1956**
Acanthocybium solandri Rohde et al., 1980

Heteromicrocotylidae

***Heteromicrocotyla australiensis* Rohde, 1977**
Carangoides fulvoguttatus Barton et al., 2009

***Heteromicrocotyloides megaspinosus* Barton, Beaufrère, Justine & Whittington, 2009**
Carangoides fulvoguttatus Barton et al., 2009

Hexabothriidae

Hexabothriidae, unidentified
Squalus melanurus Justine, 2009a

Microcotylidae

***Polylabris sillaginae* (Woolcock, 1936) Dillon, Hargis & Harrises, 1983**
Sillago sihama Hayward, 1996

Microcotylidae, unidentified species
Gymnocranius euanus Justine, 2007e
Gymnocranius grandoculis Justine, 2007e
Lethrinus nebulosus Justine, 2007e
Nemipterus furcosus Justine & Brena, 2009
Pentapodus aureofasciatus Justine & Brena, 2009

Digenea

(alphabetical order of families and species;
20 families recorded; 105 species recorded + unidentified species)

Unidentified Family, Digenea

Diagramma pictum Baillon, 1991
Epinephelus morrhua Justine, 2008c

Acanthocolpidae

***Stephanostomum japonocasum* Durio & Manter, 1969**
Epinephelus sp. “Red Cod” Durio & Manter, 1969
Serranidae “unidentified serranid” Durio & Manter, 1969

***Stephanostomum casum* (Linton, 1910) McFarlane, 1934**
Lutjanus argentimaculatus Durio & Manter, 1969

***Stephanostomum* sp.**
Pseudobalistes fuscus Bray & Justine, 2007b

Acanthocolpidae, unidentified
Pseudobalistes fuscus Bray & Justine, 2007b

Apocreadiidae

***Sphincteristomum acollum* Oshmarin, Mamaev & Parukhin, 1961**
Pseudobalistes fuscus Bray & Justine, 2007b

***Homalometron moraveci* Bray, Justine & Cribb, 2007**
Mulloidichthys vanicolensis Bray et al., 2007

Bivesiculidae

***Bivesiculoides posterotestis* Durio & Manter, 1968**
Myctophidae “pretre” *(? Atherinomorus lacunosus ?)* Durio & Manter, 1968a

Bucephalidae

***Myorhynchus pritchardae* Durio & Manter, 1968**
Serranidae (“leche”/ loche) Durio & Manter, 1968a

***Neidhartia coronata* Durio & Manter, 1968**
Epinephelus sp. Durio & Manter, 1968a

***Prosorhynchus freitasi* Nagaty, 1937**
Epinephelus sp. Durio & Manter, 1968a

***Prosorhynchus longisaccatus* Durio & Manter, 1968**

Epinephelus cyanopodus	Bray & Justine, 2006b
Serranidae ("leche"/ loche)	Durio & Manter, 1968a

***Prosorhynchus maternus* Bray & Justine, 2006**

Epinephelus malabaricus	Bray & Justine, 2006b

***Prosorhynchus serrani* Durio & Manter, 1968**

Variola louti	Durio & Manter, 1968a

***Prosorhynchoides lamprelli* Bott & Cribb, 2005**

Caranx papuensis	Bray & Justine, 2006b

***Rhipidocotyle* sp.**

Labroides dimidiatus	Jones et al., 2003, 2004

Cryptogonimidae

***Adlardia novaecaledoniae* Miller, Bray, Goiran, Justine & Cribb, 2009**

Nemipterus furcosus	Miller et al., 2009
as *Siphoderina elongata* (Gu & Shen, 1979)	
Nemipterus furcosus	Quilichini et al., 2009a

***Lobosorchis tibaldiae* Miller & Cribb, 2005**

Lutjanus fulviflamma	Miller & Cribb, 2005

***Paracryptogonimus saccatus* Manter, 1963**

Siganus sp.	Durio & Manter, 1969

***Retrovarium* (species not indicated)**

Lutjanus fulviflamma	Miller & Cribb, 2007

***Siphoderina catalae* (Durio & Manter, 1969) Miller & Cribb, 2008**

Lutjanus bohar ? ("anglais")	Durio & Manter, 1969

***Siphoderina longitestis* (Durio & Manter, 1969) Miller & Cribb, 2008**

Lutjanus bohar ? ("anglais")	Durio & Manter, 1969

***Siphoderina paracatalae* Durio & Manter, 1969**

Lutjanus bohar ? ("anglais")	Durio & Manter, 1969

***Siphoderina provitellosus* (Durio & Manter, 1969) Miller & Cribb, 2008**

Lutjanus fulvus	Durio & Manter, 1969

***Siphoderina testitactus* (Durio & Manter, 1969) Miller & Cribb, 2008**

Lutjanus bohar ? ("anglais")	Durio & Manter, 1969

Didymozoidae

Didymozoidae unidentified spp. (adults)

Epinephelus cyanopodus	Sigura & Justine, 2008
Epinephelus maculatus	Justine, 2007c
Lethrinus harak	Rascalou & Justine, 2007
Lethrinus rubrioperculatus	Justine, 2007e

Didymozoidae unidentified spp. (juveniles)

Parupeneus multifasciatus	Bray & Justine, 2008b

Didymozoidae Larva sp. A

Apogon coccineus (recruiting larvae)	Cribb et al., 2000
Bleniidae sp (recruiting larvae)	Cribb et al., 2000
Pseudogramma sp. (recruiting larvae)	Cribb et al., 2000
Scorpaenidae Gen. sp. (recruiting larvae)	Cribb et al., 2000
Synodontidae Gen. sp. (recruiting larvae)	Cribb et al., 2000

Didymozoidae Larva sp. B

Apogon coccineus (recruiting larvae)	Cribb et al., 2000
Bothus pantherinus (recruiting larvae)	Cribb et al., 2000

Didymozoidae Larva sp. C

Synodontidae Gen. sp. 1 (recruiting larvae)	Cribb et al., 2000

Didymozoidae Larva sp. D

Labridae Gen. sp. (recruiting larvae)	Cribb et al., 2000
Thalassoma sp. (recruiting larvae)	Cribb et al., 2000

Didymozoidae Larva sp. E

Apogon coccineus (recruiting larvae)	Cribb et al., 2000

Didymozoidae Larva sp. F

Apogon coccineus (recruiting larvae)	Cribb et al., 2000

Didymozoidae Larva sp. G

Labridae Gen. sp. (recruiting larvae)	Cribb et al., 2000
Thalassoma sp. (recruiting larvae)	Cribb et al., 2000

Fellodistomatidae

***Tergestia clonacantha* Manter, 1963**

Hemiramphus sp.	Durio & Manter, 1968a

Gorgoderidae

***Phyllodistomum* sp.**

Parupeneus multifasciatus	Bray & Justine, 2008b

Gyliauchenidae

***Affecauda salacia* Hall & Cribb, 2004**

Zebrasoma veliferum	Hall & Cribb, 2004b

***Gyliauchen papillatus* (Goto & Matsudaira, 1918) Goto, 1919**

Siganus sp.	Durio & Manter, 1969

***Ptychogyliauchen thistilbardi* Hall & Cribb, 2004**

Siganus argenteus	Hall & Cribb, 2004a
Siganus canaliculatus	Hall & Cribb, 2004a
Siganus corallinus	Hall & Cribb, 2004a
Siganus doliatus	Hall & Cribb, 2004a
Siganus spinus	Hall & Cribb, 2004a

Haploporidae

***Atractotrema sigani* Durio & Manter, 1969**

Siganus sp.	Durio & Manter, 1969

***Hapladena tanyorchis* Manter & Pritchard, 1961**

Naso sp. "unicorn fish"	Durio & Manter, 1969

***Isorchis parvus* Durio & Manter, 1969**

Chanos chanos	Durio & Manter, 1969

Haplosplanchnidae

***Hymenocotta mulli* Manter, 1961**

"Mullet" Mugilidae	Durio & Manter, 1968a

HEMIURIDAE

***Dichadena obesa* (Manter, 1961) Manter, 1969**
Strongylura leiura ? Manter, 1969

***Erilepturus hamati* (Yamaguti, 1934)**
[as *H. tiegsi* Woolcock, 1935 in Manter]
Epinephelus cyanopodus Manter, 1969; Sigura & Justine, 2008
Epinephelus sp. Manter, 1969
Serranidae “mottled grouper” Manter, 1969

Hemiuridae (immature unidentifiable)
Bleniidae sp. (recruiting larvae) Cribb et al., 2000

***Ectenurus* sp.**
Pseudogramma sp. (recruiting larvae) Cribb et al., 2000
Synodontidae Gen. sp. 2 (recruiting larvae) Cribb et al., 2000

***Erilepturus* sp. immature**
Synodontidae Gen. sp. 1 (recruiting larvae) Cribb et al., 2000

***Hysterolecitha sigani* Manter, 1969**
Siganus sp. Manter, 1969

***Hysterolecithoides frontilatus* (Manter, 1969) Yamaguti, 1971**
Siganus doliatus Bray & Cribb, 2000b

***Lecithaster testilobatus* Manter, 1969**
Scarus (= Callyodon) sp. Manter, 1969

***Lecithochirium cirrhiti* (Manter & Pritchard, 1960) Yamaguti, 1970**
Neoniphon sammara Moravec & Justine, 2009b
Sargocentron ensifer Moravec & Justine, 2009b
Sargocentron spiniferum Moravec & Justine, 2009b

***Lecithochirium magnaporum* Manter, 1940**
Lethrinus miniatus Manter, 1969
Epinephelus sp. Manter, 1969

***Lecithochirium polynemi* Chauhan, 1945**
Lutjanus fulvus Manter, 1969

***Lecithochirium* sp.**
Parupeneus multifasciatus Bray & Justine, 2008b

***Lecithocladium aegyptensis* Fischtal & Kuntz, 1963**
Scombridae “mackerel” Manter, 1969

***Parahemiurus merus* (Linton, 1910) Woolcock, 1935**
Priacanthus hamrur Bray & Cribb, 2005

***Quadrifoliovarium pritchardae* Yamaguti, 1965**
Naso sp. “unicorn fish” Manter, 1969

***Theletrum frontilatum* Manter, 1969**
Siganus sp. Manter, 1969

LECITHASTERIDAE
Aponurus chelebesoi Bray & Cribb, 2000a
Chaetodon auriga Bray & Cribb, 2000a
Chaetodon citrinellus Bray & Cribb, 2000a
Chaetodon ephippium Bray & Cribb, 2000a
Chaetodon flavirostris Bray & Cribb, 2000a
Chaetodon lineolatus Bray & Cribb, 2000a
Chaetodon melannotus Bray & Cribb, 2000a
Chaetodon mertensii Bray & Cribb, 2000a
Chaetodon pelewensis Bray & Cribb, 2000a
Coradion altivelis Bray & Cribb, 2000a
Forcipiger flavissimus Bray & Cribb, 2000a
Heniochus acuminatus Bray & Cribb, 2000a
Heniochus acuminatus Bray & Cribb, 2000a
Heniochus chrysostomus Bray & Cribb, 2000a
Heniochus monoceros Bray & Cribb, 2000a
Siganus doliatus Bray & Cribb, 2000a

***Aponurus laguncula* Loos, 1907**
Aluterus monoceros Quilichini et al., 2009b

LEPOCREADIIDAE

***Holorchis castex* Bray & Justine, 2007**
Diagramma pictum Bray & Justine, 2007a

***Holorchis plectorhynchi* Durio & Manter, 1968**
Lethrinus miniatus Durio & Manter, 1968b
Plectorhinchus goldmani Durio & Manter, 1968b

***Hypocreadium toombo* Bray & Justine, 2006**
Pseudobalistes fuscus Bray & Justine, 2006a

***Hypocreadium patellare* Yamaguti, 1938**
Sufflamen fraenatum Bray et al., 2009a

***Intusatrium robustum* Durio & Manter, 1968**
Bodianus loxozonus Bray & Justine, 2006a
Bodianus perditio Bray & Justine, 2006a; Durio & Manter, 1968a

***Postlepidapedon secundum* (Durio & Manter, 1968)**
Scaridae? “Brown-blotched parrot fish” [erroneous] Durio & Manter, 1968b
Choerodon graphicus Bray & Justine, 2006a

***Lepidapedoides dollfusi* (Durio & Manter, 1968) Bray, Cribb & Barker, 1996**
as *Neolepidapedon dollfusi* Durio & Manter, 1968
Epinephelus sp. “Red Cod” Durio & Manter, 1968b
Epinephelus sp. “Spotted Grouper” Durio & Manter, 1968b

***Lepidapedoides angustus* Bray, Cribb & Barker, 1996**
Epinephelus fasciatus Bray & Justine, 2006a
as *L. “kerapu”;* see Justine, 2007a
Epinephelus merra Rigby et al., 1997

***Lobatocreadium exiguum* (Manter, 1963) Madhavi, 1972**
Pseudobalistes fuscus Bray & Justine, 2006a

***Stegodexamene anguillae* Macfarlane, 1951**
Anguilla obscura Moravec & Justine, 2007c
Anguilla reinhardtii Moravec & Justine, 2007c

Lepocreadiidae, unidentified
Pseudobalistes fuscus Bray & Justine, 2007b

Microscaphidiidae

***Hexangium sigani* Goto & Ozaki, 1929**

Lutjanus fulvus Durio & Manter, 1968a
Siganus sp. Durio & Manter, 1968a

Monorchiidae

***Cableia balistidicola* Bray, Justine & Cribb, 2009**

Abalistes stellatus Bray et al., 2009b
Pseudobalistes fuscus Bray et al., 2009b
as *Cableia* sp.
Pseudobalistes fuscus Bray & Justine, 2007b

***Hysterorchis vitellosus* Durio & Manter, 1968**

Plectorhinchus sp. Durio & Manter, 1968a

***Lasiotocus longitestis* Durio & Manter, 1968**

Plectorhinchus sp. (“loche castex”) Durio & Manter, 1968a

***Lasiotocus plectorhynchi* (Yamaguti, 1934)**

Diagramma pictum Bray & Justine, 2007a

Opecoelidae

***Allopodocotyle epinepheli* (Yamaguti, 1942)**

Epinephelus cyanopodus Bray & Justine, 2007b
Epinephelus fasciatus Bray & Justine, 2007b
Epinephelus merra Bray & Justine, 2007b

***Allopodocotyle serrani* (Yamaguti, 1952) Pritchard, 1966**

Serranidae (“leche”/ loche) Durio & Manter, 1968b

***Cainocreadium epinepheli* (Yamaguti, 1934)**

Epinephelus coeruleopunctatus Bray & Justine, 2007b
Epinephelus cyanopodus Sigura & Justine, 2008
Epinephelus fasciatus Bray & Justine, 2007b
Variola louti Bray & Justine, 2007b

***Cainocreadium* sp.**

Epinephelus malabaricus Justine & Sigura, 2007

***Hamacreadium diacopae* Nagaty & Abdel Aal, 1962**

Lethrinus nebulosus ? (“bec de cane”)Durio & Manter, 1968b

***Hamacreadium mutabile* (Linton, 1910)**

Lethrinus miniatus Durio & Manter, 1968b
Lutjanus amabilis Durio & Manter, 1968b
Lutjanus bohar ? (““anglais”) Durio & Manter, 1968b
Lutjanus fulviflamma Bray & Justine, 2007b
Lutjanus kasmira Bray & Justine, 2007b

***Choanostoma secundum* Durio & Manter, 1968**

Lutjanus vitta Durio & Manter, 1968b
Plectorhinchus sp. Durio & Manter, 1968b

***Helicometra epinepheli* Yamaguti, 1934**

Epinephelus fasciatus Bray & Justine, 2007b
Epinephelus merra Bray & Justine, 2007b

***Helicometra fasciata* (Rudolphi, 1819) Odhner, 1902**

Epinephelus merra Rigby et al., 1997
Epinephelus sp. “Red Cod” Durio & Manter, 1968b

***Macvicaria jagannathi* (Gupta & Singh, 1985) Bijukumar, 1997**

Nemipterus furcosus Bray & Justine, 2009b

***Neolebouria blatta* Bray & Justine, 2009**

Etelis carbunculus Bray & Justine, 2009a
Pristipomoides argyrogrammicus
Bray & Justine, 2009a

***Neolebouria lineatus* Aken’Ova & Cribb, 2001**

Nemipterus furcosus Bray & Justine, 2009b

***Orthodena tropica* Durio & Manter, 1968**

Lethrinus lentjan Bray & Justine, 2007b
Lethrinus nebulosus ? (“bec de cane”)
Durio & Manter, 1968b

***Pacificreadium serrani* Durio & Manter, 1968**

Epinephelus sp. Durio & Manter, 1968b
Plectropomus leopardus Bray & Justine, 2007b

***Pseudoplagioporus interruptus* Durio & Manter, 1968**

Lethrinus nebulosus ? (“bec de cane”) Durio & Manter, 1968b
Lethrinus rubrioperculatus Bray & Justine, 2007b;
Justine, 2007e

***Pseudoplagioporus lethrini* Yamaguti, 1938**

Lethrinus nebulosus ? (“bec de cane”)
Durio & Manter, 1968b

***Pseudopycnadena tendu* Bray & Justine, 2007**

Pseudobalistes fuscus Bray & Justine, 2007b

Unidentified Opecoelidae

Lethrinus harak Rascalou & Justine, 2007
Lethrinus nebulosus Rascalou & Justine, 2007

Sanguinicolidae

***Cardicola chaetodontis* Yamaguti, 1970**

Chaetodon lineolatus Nolan & Cribb, 2006

Syncoeliidae

***Syncoelium filiferum* (Sars, 1885) Odhner, 1911**

Katsuwonus pelamis Rohde et al., 1980

Zoogonidae

***Deretrema combesae* Bray & Justine, 2008**

Parupeneus multifasciatus Bray & Justine, 2008b

***Deretrema combesorum* Bray & Justine, 2008**

Parupeneus multifasciatus Bray & Justine, 2008b

***Diphtherostomum tropicum* Durio & Manter, 1968**

Lethrinus nebulosus ? Durio & Manter, 1968a

***Dupliciporia lanterna* Bray & Justine, 2008**

Priacanthus hamrur Bray & Justine, 2008a

***Lecithostaphylus nitens* (Linton, 1898)**

Tylosurus crocodilus Bray & Justine, 2008a

Parvipyrum acanthuri **Pritchard, 1963**
Acanthurus blochii Bray & Justine, 2008a

Zoogonus pagrosomi **Yamaguti, 1939**
Lethrinus atkinsoni Bray & Justine, 2008a
Lethrinus genivittatus Bray & Justine, 2008a

CESTODA BOTHRIOCEPHALIDEA

1 family recorded; 2 species recorded

BOTHRIOCEPHALIDAE

Bothriocephalus celineae **Kuchta, Scholz & Justine, 2009 [adult]**
Cephalopholis aurantia × *C. spiloparaea* Kuchta et al., 2009a
as **'adult pseudophyllidean cestode'**
Cephalopholis aurantia × *C. spiloparaea* Randall & Justine, 2008a

Oncodiscus sauridae **Yamaguti, 1934 [adult]**
Saurida nebulosa Kuchta et al., 2009b
Saurida undosquamis Kuchta et al., 2009b

CESTODA TETRAPHYLLIDEA

At least 1 family recorded; 6 species recorded + unidentified species

ONCHOBOTHRIIDAE

Acanthobothrium aetiobatis **(Shipley, 1900)**
Aetobatus narinari Baer & Euzet, 1962

UNIDENTIFIED FAMILY

Scolex polymorphus **(= tetraphyllidean metacestodes)**
Epinephelus merra Rigby et al., 1997

Tetraphyllidea larvae
Epinephelus cyanopodus Sigura & Justine, 2008
Epinephelus morrhua Justine, 2008c

Metacestode
Synodontidae Gen. sp. 3 (recruiting larvae) Cribb et al., 2000

Tetraphyllidea Metacestode
Thalassoma sp. (recruiting larvae) Cribb et al., 2000

Unidentified Tetraphyllidea, adults
Centrophorus sp. Beveridge & Justine, 2006
Squalus megalops Beveridge & Justine, 2006
Squalus melanurus Beveridge & Justine, 2006

CESTODA TRYPANORHYNCHA

5 families recorded; 20 species recorded + unidentified species

FAMILY UNIDENTIFIED

Trypanorhyncha, unidentified [larva]
Epinephelus merra Rigby et al., 1997
Epinephelus morrhua Justine, 2008c

GILQUINIIDAE

Gilquinia robertsoni **Beveridge, 1990 [adult]**
Squalus megalops Beveridge & Justine, 2006

Gilquinia minor **Beveridge & Justine, 2006 [adult]**
Centrophorus sp. Beveridge & Justine, 2006

Gilquinia **sp. [adult]**
Squalus melanurus Beveridge & Justine, 2006

Sagittirhynchus aculeatus **Beveridge & Justine, 2006 [adult]**
Centrophorus sp. Beveridge & Justine, 2006

Vittirhynchus squali **Beveridge & Justine, 2006 [adult]**
Squalus melanurus Beveridge & Justine, 2006

GYMNORHYNCHIDAE

Chimaerarhynchus rougetae **Beveridge & Campbell, 1989 [adult]**
Squalus cf. *megalops* Olson et al., 2009; Palm et al., 2009

LACISTORHYNCHIDAE

Floriceps minacanthus **Campbell & Beveridge, 1987 [larva]**
Cephalopholis urodeta Justine, 2008a
Epinephelus cyanopodus Sigura & Justine, 2008

Hornelliella annandalei **Hornell, 1912 [adult]**
Stegostoma fasciatum Beveridge & Justine, 2007b

Paragrillotia apecteta **Beveridge & Justine, 2007 [adult]**
Hexanchus nakamurai Beveridge & Justine, 2007a

Paragrillotia spratti **(Campbell & Beveridge, 1993) Beveridge & Justine, 2007 [adult]**
Negaprion acutidens Beveridge & Justine, 2007a

Pseudogilquinia pillersi **(Southwell, 1929) Palm, 2004 [larva]**
Epinephelus coioides Beveridge et al., 2007

Pseudolacistorhynchus heroniensis **(Sakanari, 1989) Palm, 1995**
Epinephelus cyanopodus [larva] Sigura & Justine, 2008
Stegostoma fasciatum [adult] Beveridge & Justine, 2007b

Pseudolacistorhynchus nanus **Beveridge & Justine, 2007 [adult]**
Stegostoma fasciatum Beveridge & Justine, 2007b

OTOBOTHRIIDAE

Otobothrium curtum **(Linton, 1909) Dollfus, 1942 [adult]**
Galeocerdo cuvier Beveridge & Justine, 2007c

Otobothrium parvum **Beveridge & Justine, 2007 [adult]**
Carcharhinus amblyrhynchos Beveridge & Justine, 2007c
Triaenodon obesus Beveridge & Justine, 2007c

Otobothrium **sp. [larva]**
Lethrinus rubrioperculatus Justine, 2007e

PSEUDOTOBOTHRIIDAE

Pseudotobothrium dipsacum **(Linton, 1897) [larva]**
Epinephelus malabaricus Justine & Sigura, 2007

Tentaculariidae

Mixonybelinia southwelli **(Palm & Walter, 1999) Palm, 1999 [adult]**
Stegostoma fasciatum Beveridge & Justine, 2007b

Nybelinia syngenes **(Pintner, 1928) Palm, 2004 [larva]**
Dendrochirus zebra Palm, 2004
as *Nybelinia aequidentata* [larva]
Dendrochirus zebra Palm & Beveridge, 2002

Nybelinia **sp. [larva]**
Lethrinus rubrioperculatus Justine, 2007e

Nematoda

(alphabetical order of families and species; 10 families recorded; 43 species recorded + unidentified species)

Unidentified Nematoda
Epinephelus morrhua Justine, 2008c

Anisakidae

Raphidascaris (Ichtyiascaris) nemipteri **Moravec & Justine, 2005 [adult]**
Nemipterus furcosus Moravec & Justine, 2005a

Hysterothylacium cenaticum **(Bruce & Cannon, 1989) Moravec & Justine, 2005 [adult]**
Tetrapturus audax Moravec & Justine, 2005a

Terranova scoliodontis **(Baylis, 1931) Johnston & Mawson, 1945 [adult]**
Galeocerdo cuvier Moravec & Justine, 2006b

Terranova **sp. [larva]**
Epinephelus cyanopodus Sigura & Justine, 2008

Camallanidae

Unidentified Camallanidae
Diagramma pictum Baillon, 1991

Camallanus carangis **Olsen, 1954**
Nemipterus furcosus Moravec et al., 2006a
Parupeneus ciliatus Moravec et al., 2006a
Upeneus vittatus Moravec et al., 2006a

Camallanus cotti **Fujita, 1927**
Awaous guamensis Moravec & Justine, 2006a
Kuhlia marginata **[larva]** Moravec & Justine, 2006a

Procamallanus (Spirocamallanus) longus **Moravec, Justine & Rigby, 2006**
Zebrasoma scopas Moravec et al., 2006a

Procamallanus (Spirocamallanus) variolae **Moravec, Justine & Rigby, 2006**
Epinephelus fasciatus Moravec et al., 2006a
Variola albimarginata Moravec et al., 2006a

Procamallanus (Procamallanus) pacificus **Moravec, Justine, Würtz, Taraschewski & Sasal, 2006**
Anguilla obscura Moravec et al., 2006b
Anguilla reinhardtii Moravec et al., 2006b

Procamallanus (Spirocamallanus) **sp. 1**
Parapercis hexophtalma Moravec et al., 2006a

Procamallanus (Spirocamallanus) **sp. 2**
Lethrinus ravus Moravec et al., 2006a

Procamallanus (Spirocamallanus) **sp. 3**
Scolopsis bilineata Moravec et al., 2006a

Procamallanus **sp.**
Epinephelus cyanopodus Sigura & Justine, 2008

Capillariidae

Capillariidae Gen. sp. 7 of Moravec, 2001
Siganus doliatus Moravec, 2001

Capillariidae Gen. sp. 8 of Moravec, 2001
Naso unicornis Moravec, 2001

Cucullanidae

Cucullanus bourdini **Petter & Le Bel, 1992**
Aprion virescens Petter & Le Bel, 1992
Pristipomoides filamentosus Petter & Le Bel, 1992
Pristipomoides flavipinnis Petter & Le Bel, 1992

Cucullanidae, unidentified
Bodianus perditio Lim & Justine, 2007

Cystidicolidae

Ascarophis (Dentiascarophis) adioryx **Machida, 1981**
Neoniphon sammara Moravec & Justine, 2009b
Sargocentron spiniferum Moravec & Justine, 2009b

Ascarophis (Similarascarophis) richeri **Moravec & Justine, 2007**
Hoplichthys citrinus Moravec & Justine, 2007a

Ctenascarophis lesteri **Crites, Overstreet & Maung, 1993**
Katsuwonus pelamis Crites et al., 1993

Metabronema magnum **(Taylor, 1925) Yorke & Maplestone, 1926**
Gnathanodon speciosus Moravec & Justine, 2007b

Spinitectus (Paraspinitectus) **sp.**
Albula glossodonta Moravec & Justine, 2009b

Gnathostomatidae

Echinocephalus sinensis **Ko, 1975**
Aetobatus narinari Moravec & Justine, 2006b

Echinocephalus overstreeti **Deardoff & Ko, 1983**
Urogymnus asperimmus [not *Taeniura meyeni*, erroneous]
Moravec & Justine, 2006b

Guyanemidae

Ichthyofilaria novaecaledoniensis **Moravec & Justine, 2009**
Hoplichthys citrinus Moravec & Justine, 2009a

Philometridae

Philometra cyanopodi Moravec & Justine, 2008

Epinephelus cyanopodus	Moravec & Justine, 2008

as _Ph. lateolabracis_ (Yamaguti, 1935)

Epinephelus cyanopodus	Moravec & Justine, 2005b

Philometra dentigubernaculata Moravec & Justine, 2009

Tylosurus crocodilus	Moravec & Justine, 2009a

Philometra fasciati Moravec & Justine, 2008

Epinephelus fasciatus	Moravec & Justine, 2008

as _Ph. lateolabracis_ (Yamaguti, 1935)

Epinephelus fasciatus	Moravec & Justine, 2005b

Philometra lagocephali Moravec & Justine, 2008

Lagocephalus sceleratus	Moravec & Justine, 2008

Philometra lethrini Moravec & Justine, 2008

Lethrinus genivittatus	Moravec & Justine, 2008, 2009a
Lethrinus miniatus	Moravec & Justine, 2009a
Lethrinus variegatus	Moravec & Justine, 2009a

Philometra ocularis Moravec, Ogawa, Suzuki, Miyazaki & Donai, 2002

Epinephelus areolatus	Moravec & Justine, 2008, 2009a
Epinephelus coioides	Moravec & Justine, 2005b
Epinephelus cyanopodus	Moravec & Justine, 2005b
Epinephelus rivulatus	Moravec & Justine, 2005b
Variola louti	Moravec & Justine, 2005b

Philometra priacanthi Moravec & Justine, 2009

Priacanthus hamrur	Moravec & Justine, 2009a

Philometra tenuicauda Moravec & Justine, 2009

Lagocephalus sceleratus	Moravec & Justine, 2009a

Philometra sp. Moravec & Justine, 2008

Lutjanus vitta	Moravec & Justine, 2008

Rhabdochonidae

Unidentified Rhabdochonidae

as _Huffmanela balista,_ female [erroneous]

Abalistes stellatus	Justine, 2007b

Trichosomoididae

Huffmanela balista Justine, 2007

Abalistes stellatus	Justine, 2007b

Huffmanela branchialis Justine, 2004

Nemipterus furcosus	Justine, 2004

Huffmanela filamentosa Justine, 2004

Gymnocranius grandoculis	Justine, 2004

Huffmanela lata Justine, 2005

Carcharhinus amblyrhynchos	Justine, 2005a

Huffmanela longa Justine, 2007

Gymnocranius grandoculis	Justine, 2007b

Huffmanela ossicola Justine, 2004

Bodianus busellatus	Justine, 2007b
Bodianus loxozonus	Justine, 2004, 2007b
Bodianus perditio	Justine, 2007b; Lim & Justine, 2007

Huffmanela sp.

Pentapodus aureofasciatus	Justine, 2004, 2007b

Copepoda

(alphabetical order of families and species)

The compilation by Boxshall & Huys (2007) does not indicate hosts. This paper was based in part on collection by the present author, so additional host information was indicated here as "present paper".
13 families recorded; 61 species recorded + unidentified species

Unidentified Family

Copepoda, unidentified larvae

Abalistes filamentosus	Randall & Justine, 2008b
Cephalopholis aurantia x *C. spiloparaea* (hybrid)	Randall & Justine, 2008a
Cephalopholis sonnerati	Justine, 2007d
Lethrinus xanthochilus	Justine, 2007e

Copepoda, unidentified adults

Cephalopholis urodeta	Justine, 2008a

Bomolochidae

Pumiliopes capitulatus Cressey & Boyle, 1973

No host indicated; present paper: *Rastrelliger kanagurta*	Boxshall & Huys, 2007

Caligidae

Alanlewisia fallolunulus (Lewis, 1967) Boxshall, 2008

Naso unicornis	Boxshall, 2008

Alebion carchariae Krφyer, 1863

No host indicated; present paper: *Galeocerdo cuvier*	Boxshall & Huys, 2007

Alebion gracilis Wilson, 1905

No host indicated; present paper: *Stegostoma fasciatum*	Boxshall & Huys, 2007

Anuretes justinei Venmathi Maran, Ohtsuka & Boxshall, 2008

Plectorhinchus lineatus	Venmathi Maran et al., 2008

Avitocaligus assurgericola Boxshall & Justine, 2005

Assurger anzac	Boxshall & Justine, 2005

Caligus bonito Wilson, 1905

Katsuwonus pelamis	Rohde et al., 1980

Caligus confusus Pillai, 1961

No host indicated; present paper: *Caranx papuensis*	Boxshall & Huys, 2007

Caligus cordyla Pillai, 1963

No host indicated; present paper: *Carangoides fulvoguttatus*	Boxshall & Huys, 2007

***Caligus coryphaenae* Steenstrup & Lütken, 1861**
No host indicated; present paper: *Coryphaena hippurus*
Boxshall & Huys, 2007

***Caligus infestans* Heller, 1868**
No host indicated; present paper:
Scomberomorus commerson Boxshall & Huys, 2007

***Caligus isonyx* Steenstrup & Lütken, 1861**
No host indicated; present paper: *Sphyraena flavicauda*
Boxshall & Huys, 2007

***Caligus lobodes* (Wilson, 1911) Pillai, 1985**
No host indicated; present paper: *Sphyraena barracuda*
Boxshall & Huys, 2007

***Caligus lethrinicola* Boxshall & El-Rashidy, 2009**
Lethrinus rubrioperculatus Boxshall & El-Rashidy, 2009
as *Caligus* cf. *mauritanicus* Brian, 1924
Lethrinus rubrioperculatus Boxshall & Huys, 2007; Justine, 2007e

***Caligus novocaledonicus* Kabata, 1968**
No host indicated Boxshall & Huys, 2007

***Caligus productus* Dana, 1852**
Acanthocybium solandri Rohde et al., 1980
Katsuwonus pelamis Rohde et al., 1980

***Gloiopotes hygomianus* Steenstrup & Lütken, 1861**
No host indicated; present paper:
Acanthocybium solandri Boxshall & Huys, 2007

***Gloiopotes watsoni* Kirtisinghe, 1934**
No host indicated; present paper: *Tetrapturus audax*
Boxshall & Huys, 2007

***Lepeophtheirus* sp.**
Bodianus perditio Lim & Justine, 2007
Epinephelus malabaricus Justine & Sigura, 2007

***Paralebion elongatus* Wilson, 1911**
No host indicated; present paper:
Triaenodon obesus Boxshall & Huys, 2007

***Pseudanuretes fortipedis* Kabata, 1965**
No host indicated; present paper:
Pomacanthus semicirculatus Boxshall & Huys, 2007

DISSONIDAE
***Dissonus excavatus* Boxshall, Lin, Ho, Ohtsuka, Venmathi Maran & Justine, 2008**
Bodianus perditio Boxshall et al., 2008
as *Dissonus* sp.
Bodianus perditio Lim & Justine, 2007
Macolor niger Boxshall et al., 2008

***Dissonus heronensis* Kabata, 1966**
Pseudobalistes fuscus Boxshall et al., 2008

***Dissonus manteri* Kabata, 1966**
Epinephelus cyanopodus Boxshall et al., 2008
Epinephelus maculatus Boxshall et al., 2008; Journo & Justine, 2006
Plectropomus laevis Boxshall et al., 2008
Plectropomus leopardus Boxshall et al., 2008; Justine & Euzet, 2006

EUDACTYLINIDAE
***Nemesis robusta* (van Beneden, 1851) Heller, 1868**
No host indicated; present paper:
Carcharhinus amblyrhynchos Boxshall & Huys, 2007

HATSCHEKIIDAE
***Hatschekia balistae* Nunes-Ruivo, 1954**
No host indicated; present paper:
Abalistes stellatus Boxshall & Huys, 2007
No host indicated; present paper:
Balistoides conspicillum Boxshall & Huys, 2007
No host indicated; present paper:
Pseudobalistes fuscus Boxshall & Huys, 2007

***Hatschekia cernae* Goggio, 1905**
No host indicated Boxshall & Huys, 2007
Epinephelus cyanopodus Sigura & Justine, 2008

***Hatschekia* cf. *plectropomi* Ho & Dojiri, 1978**
No host indicated; present paper:
Plectropomus laevis Boxshall & Huys, 2007
No host indicated; present paper:
Plectropomus leopardus Boxshall & Huys, 2007; Justine & Euzet, 2006

***Hatschekia sphyraeni* Pillai, 1964**
No host indicated; present paper:
Sphyraena forsteri Boxshall & Huys, 2007

***Hatschekia* sp.**
Bodianus perditio Lim & Justine, 2007
Epinephelus cyanopodus (2 species) Sigura & Justine, 2008
Epinephelus fasciatus Justine, 2005b
Epinephelus maculatus (2 species) Journo & Justine, 2006
Epinephelus malabaricus Justine & Sigura, 2007
Lethrinus harak Rascalou & Justine, 2007
Lethrinus nebulosus Rascalou & Justine, 2007

***Laminohatschekia synaphobranchi* Boxshall,1989**
Synaphobranchus cf. *brevidorsalis* Boxshall, 1989

KROYERIIDAE
***Kroyeria dispar* Wilson, 1935**
No host indicated; present paper:
Galeocerdo cuvier Boxshall & Huys, 2007

LERNANTHROPIDAE
***Lernanthropus cadenati* Delamare-Deboutteville & Nunes-Ruivo, 1954**
No host indicated; present paper:
Elops hawaiensis Boxshall & Huys, 2007

***Lernanthropus corniger* Yamaguti, 1954**
No host indicated; present paper:
Megalaspis cordyla Boxshall & Huys 2007

***Lernanthropus tylosuri* Richiardi, 1880**
No host indicated; present paper:
Tylosurus crocodilus Boxshall & Huys, 2007

***Lernanthropus* sp.**
Bodianus perditio Lim & Justine, 2007

***Norion priacanthi* (Kirtisinghe, 1956) Ho & Kim, 2004**
No host indicated; present paper:
Priacanthus hamrur Boxshall & Huys, 2007

***Sagum epinepheli* (Yamaguti & Yamasu, 1960) Pillai & Sebastian, 1967**
No host indicated; present paper:
Epinephelus chlorostigma Boxshall & Huys, 2007
No host indicated; present paper:
Epi. coeruleopunctatus Boxshall & Huys, 2007
No host indicated; present paper:
Epinephelus cyanopodus Boxshall & Huys, 2007; Sigura & Justine, 2008
No host indicated; present paper:
Epinephelus merra Boxshall & Huys, 2007
No host indicated; present paper:
Epinephelusmorrhua Boxshall & Huys, 2007
No host indicated; present paper:
Plectropomus leopardus Boxshall & Huys, 2007
as unidentified Copepoda *Epinephelus coeruleopunctatus* Sigura et al., 2007
as ***Sagum* sp.** *Plectropomus leopardus* Justine & Euzet, 2006
as *Aethon* sp. *Epinephelus merra* Justine, 2005b

Lernaeopodidae

***Brachiella thynni* Cuvier, 1830**
Acanthocybium solandri Rohde et al., 1980

***Margolisius* cf. *abditus* Benz, Kabata & Bullard, 2000**
No host indicated; present paper:
Echeneis naucrates Boxshall & Huys, 2007

***Naobranchia* cf. *spinosa* Pearse, 1952**
No host indicated; present paper:
Selar crumenophtalmus Boxshall & Huys, 2007

***Parabrachiella appendiculata* (Heegaard, 1947) Boxshall & Halsey, 2004**
No host indicated; present paper:
Chirocentrus dorab Boxshall & Huys, 2007

Pandaridae

***Demoleus latus* Shiino, 1954**
No host indicated; present paper:
Squalus megalops Boxshall & Huys, 2007

***Dinemoura latifolia* (Steenstrup & Lütken, 1861) Yamaguti, 1963**
No host indicated; present paper:
Isurus oxyrhinchus Boxshall & Huys, 2007

***Echthrogaleus coleoptratus* (Guérin-Méneville, 1837) Steenstrup & Lütken, 1861**
No host indicated; present paper:
Prionace glauca Boxshall & Huys, 2007

***Nesippus crypturus* Heller, 1868**
No host indicated; present paper:
Galeocerdo cuvier Boxshall & Huys, 2007

***Nesippus tigris* Cressey, 1967**
No host indicated; present paper:
Galeocerdo cuvier Boxshall & Huys, 2007

***Pandarus satyrus* Dana, 1852**
No host indicated; present paper:
Prionace glauca Boxshall & Huys, 2007

***Pandarus smithii* Rathbun, 1886**
No host indicated; present paper:
Pristipomoides filamentosus Boxshall & Huys, 2007

***Pseudopandarus gracilis* Kirtisinghe, 1950**
No host indicated; present paper:
Squalus megalops Boxshall & Huys, 2007
No host indicated; present paper:
Squalus melanurus Boxshall & Huys, 2007

Pennellidae

***Lernaeolophus striatus* Wilson, 1913**
No host indicated; present paper:
Pristipomoides filamentosus Boxshall & Huys, 2007

***Sarcotretes scopeli* Jungersen, 1911**
Hymenocephalus gracilis Boxshall, 1989

Unidentified larvae
Epinephelus cyanopodus Sigura & Justine, 2008

Pseudocycnidae

***Pseudocycnus appendiculatus* Heller, 1868**
No host indicated; present paper:
Thunnus albacares Boxshall & Huys, 2007

Sphyriidae

***Lophoura cornuta* (Wilson, 1919) Yamaguti, 1963**
Synaphobranchus cf. *brevidorsalis* Boxshall, 1989

Taeniacanthidae

***Irodes sauridi* (Pillai, 1963)**
No host indicated; present paper:
Upeneus vittatus Boxshall & Huys, 2007

***Metataeniacanthus vulgaris* Cressey & Cressey, 1979**
No host indicated; present paper:
Synodus similis Boxshall & Huys 2007
No host indicated; present paper:
Synodus variegatus Boxshall & Huys, 2007

***Taeniacanthus aluteri* (Avdeev, 1977) Dojiri & Cressey, 1987**
No host indicated; present paper:
Abalistes stellatus Boxshall & Huys, 2007

***Taeniacanthus kitamakura* Yamaguti & Yamasu, 1959**
No host indicated; present paper:
Lagocephalus sceleratus Boxshall & Huys, 2007

Isopoda

(alphabetical order of families and species; 4 families recorded; 27 species recorded + unidentified species)

Unidentified Adult Isopod

Diagramma pictum Baillon, 1991

Aegidae

***Aega alazon* Bruce, 2004**
Host unknown [Deep-sea] Bruce, 2004

***Aega angustata* Whitelegge, 1901**
Hexanchus nakamurai Trilles & Justine, 2004
Squalus megalops Trilles & Justine, 2004
Squalus melanurus Trilles & Justine, 2004
as *Aega* sp. Bruce, 2007

***Aega coroo* Bruce, 2004**
No host indicated Bruce, 2007

***Aega* cf. *deshaysian*a (Milne Edwards, 1840) Schioedte & Meinert, 1879**
Squalus megalops Trilles & Justine, 2004

***Aega hamiota* Bruce, 2004**
Host unknown [Deep-sea] Bruce, 2004

***Aega kixalles* Bruce, 2004**
Host unknown [Deep-sea] Bruce, 2004

***Aega monophthalma* Johnston, 1834**
No host indicated Bruce, 2007

***Aega musorstom* Bruce, 2004**
Synagrops sp. [Deep-sea] Bruce, 2004

***Aega plebeia* Hansen, 1897**
No host indicated Bruce, 2007

***Aega rickbruscai* Bruce, 2004**
Host unknown [Deep-sea] Bruce, 2004

***Aega vigilans* Haswell, 1881**
No host indicated Bruce, 2007

***Aega webbi* (Guérin-Méneville, 1836) Schioedte & Meinert, 1879**
Squalus megalops Trilles & Justine, 2004
as *Aega urotoma* Barnard, 1914 Bruce, 2007

***Aega* sp. of Bruce, 2004**
Host unknown Bruce, 2004

***Syscenus latus* Richardson, 1909**
No host indicated Bruce, 2007

***Syscenus moana* Bruce, 2005**
No host indicated Bruce, 2007

Corallanidae

***Argathona macronema* (Bleeker, 1857) Monod, 1975**
Plectropomus laevis Justine & Euzet, 2006

***Argathona rhinoceros* (Bleeker, 1857) Monod, 1975**
Epinephelus cyanopodus Sigura & Justine, 2008
Plectropomus leopardus Justine & Euzet, 2006

Cymothoidae

***Anilocra australis* Schioedte & Meinert, 1881**
No host indicated Bruce, 2007

***Anilocra gigantea* (Herklots, 1870) Schioedte & Meinert, 1881**
Etelis carbunculus Trilles, 1972

***Anilocra longicauda* Schioedte & Meinert, 1881**
No host indicated Bruce, 2007

***Ceratothoa carinata* (Bianconi, 1869) Schioedte & Meinert, 1883**
'Lutjanus amabilis' [unknown in FishBase] Trilles, 1972

***Ceratothoa impressa* (Say, 1818) Richardson, 1905**
"Espadon (Swordfish)" Trilles 1972

***Elthusa arnoglossi* Trilles & Justine, 2006**
Arnoglossus sp. Trilles & Justine, 2006

***Elthusa parabothi* Trilles & Justine, 2004**
Parabothus kiensis Trilles & Justine, 2004

***Nerocila excisa* (Richardson, 1901) Richardson Searle, 1914**
Grammistes sp. Trilles, 1972

Gnathiidae

Gnathiidae Gen. sp. larva
Abalistes filamentosus Randall & Justine, 2008b
Bodianus perditio Lim & Justine, 2007
Cephalopholis argus Justine, 2007d
Cephalopholis boenak Justine, 2007d
Cephalopholis sonnerati Justine, 2007d
Cephalopholis urodeta Justine, 2008a
Epinephelus cyanopodus Sigura & Justine, 2008
Epinephelus maculatus Journo & Justine, 2006
Epinephelus morrhua Justine, 2008c
Epinephelus rivulatus Hinsinger & Justine, 2006a
Hemigymnus melapterus Grutter, 1999
Lethrinus harak Rascalou & Justine, 2007
Lethrinus rubrioperculatus Justine, 2007e
Pentapodus aureofasciatus Justine & Brena, 2009
Scolopsis bilineatus Grutter, 1999

Acanthocephala

only unidentified species

Unidentified Acanthocephala
Epinephelus morrhua Justine, 2008c

Hirudinea

only unidentified species

Unidentified Hirudinea
Diagramma pictum Baillon, 1991
Lethrinus harak Rascalou & Justine, 2007

TABLE 2.

Host-Parasite list

57 families recorded with parasites; 177 species recorded with parasites

Host Unknown (or not indicated)

Copepoda *Caligus novocaledonicus* Boxshall & Huys, 2007
Isopoda *Aega alazon* Bruce, 2004
Isopoda *Aega coroo* Bruce, 2007
Isopoda *Aega hamiota* Bruce, 2004
Isopoda *Aega kixalles* Bruce, 2004
Isopoda *Aega monophthalma* Bruce, 2007
Isopoda *Aega plebeia* Bruce, 2007
Isopoda *Aega rickbruscai* Bruce, 2004
Isopoda *Aega* sp. Bruce, 2004
Isopoda *Aega vigilans* Bruce, 2007
Isopoda *Anilocra australis* Bruce, 2007
Isopoda *Anilocra gigantea* Trilles, 1972
Isopoda *Anilocra longicauda* Bruce, 2007
Isopoda *Syscenus latus* Bruce, 2007
Isopoda *Syscenus moana* Bruce, 2007

Elasmobranchii

(alphabetical order of families and species;
9 families recorded with parasites; 14 species recorded with parasites)

Carcharhinidae

***Carcharhinus amblyrhynchos* (Bleeker)**
C. Trypanorhyncha *Otobothrium parvum* Beveridge & Justine, 2007c
Nematoda *Huffmanela lata* Justine, 2005a
Copepoda *Nemesis robusta* Boxshall & Huys, 2007

***Carcharhinus plumbeus* (Nardo)**
Copepoda *Pandarus smithii* Boxshall & Huys, 2007

***Galeocerdo cuvier* (Péron & Lesueur)**
C. Trypanorhyncha *Otobothrium curtum* Beveridge & Justine, 2007c
Nematoda *Terranova scoliodontis* Moravec & Justine, 2006b
Copepoda *Alebion carchariae* Boxshall & Huys, 2007
Copepoda *Kroyeria dispar* Boxshall & Huys, 2007
Copepoda *Nesippus crypturus* Boxshall & Huys, 2007
Copepoda *Nesippus tigris* Boxshall & Huys, 2007

***Negaprion acutidens* (Rüppell)**
C. Trypanorhyncha *Paragrillotia spratti* Beveridge & Justine, 2007a

***Prionace glauca* (Linnaeus)**
Copepoda *Echthrogaleus coleoptratus* Boxshall & Huys, 2007
Copepoda *Pandarus satyrus* Boxshall & Huys, 2007

Centrophoridae

***Centrophorus* sp. [Deep-sea]**
C. Tetraphyllidea, unidentified adults Beveridge & Justine, 2006
C. Trypanorhyncha *Gilquinia minor* Beveridge & Justine, 2006
C. Trypanorhyncha *Sagittirhynchus aculeatus* Beveridge & Justine, 2006

Dasyatidae

***Urogymnus asperimmus* (Bloch & Schneider)** [not *Taeniura meyeni* Müller & Henle, erroneous]
Nematoda *Echinocephalus overstreeti* Moravec & Justine, 2006b

Hemigaleidae

***Triaenodon obesus* (Rüppell)**
C. Trypanorhyncha *Otobothrium parvum* Beveridge & Justine, 2006
Copepoda *Paralebion elongatus* Boxshall & Huys, 2007

Hexanchidae

***Hexanchus nakamurai* Teng [Deep-sea]**
C. Trypanorhyncha *Paragrillotia apecteta* Beveridge & Justine, 2007a
Isopoda *Aega angustata* Trilles & Justine, 2004

Lamnidae

***Isurus oxyrhinchus* Rafinesque**
Copepoda *Dinemoura latifolia* Boxshall & Huys, 2007

Myliobatidae

***Aetobatus narinari* (Euphrasen)**
Monopisthocotylea *Clemacotyle australis* Marie & Justine, 2005
Monopisthocotylea *Decacotyle elpora* Marie & Justine, 2005
Monopisthocotylea *Decacotyle octona* Marie & Justine, 2005
Monopisthocotylea *Thaumatocotyle pseudodasybatis* Marie & Justine, 2005, 2006
C. Tetraphyllidea *Acanthobothrium aetiobatis* Baer & Euzet, 1962
Nematoda *Echinocephalus sinensis* Moravec & Justine, 2006b

Squalidae

***Squalus megalops* Macleay [Deep-sea]**
C. Tetraphyllidea, unidentified adults Beveridge & Justine, 2006
C. Trypanorhyncha *Gilquinia robertsoni* Beveridge & Justine, 2006
Copepoda *Demoleus latus* Boxshall & Huys, 2007
Copepoda *Pseudopandarus gracilis* Boxshall & Huys, 2007
Isopoda *Aega angustata* Trilles & Justine, 2004
Isopoda *Aega webbi* Trilles & Justine, 2004
as *Aega urotoma* Bruce, 2007
Isopoda *Aega* cf. *deshaysiana* Trilles & Justine, 2004

***Squalus* cf. *megalops* [Deep-sea]**
C. Trypanorhyncha *Chimaerarhynchus rougetae* Olson et al., 2010; Palm et al., 2009

***Squalus melanurus* Fourmanoir & Rivaton [Deep-sea]**
Monopisthocotylea *Triloculotrema chisholmae* Justine, 2009a
Polyopisthocotylea Hexabothriidae, unidentified Justine, 2009a
C. Tetraphyllidea, unidentified adults Beveridge & Justine, 2006
C. Trypanorhyncha *Gilquinia* sp. Beveridge & Justine, 2006
C. Trypanorhyncha *Vittirhynchus squali* Beveridge & Justine, 2006
Copepoda *Pseudopandarus gracilis* Boxshall & Huys, 2007
Isopoda *Aega angustata* Trilles & Justine, 2004

STEGOSTOMATIDAE
***Stegostoma fasciatum* (Hermann)**
C. Trypanorhyncha *Hornelliella annandalei* Beveridge & Justine, 2007b
C. Trypanorhyncha *Mixonybelinia southwelli* Beveridge & Justine, 2007b
C. Trypanorhyncha *Pseudolacistorhynchus heroniensis* Beveridge & Justine, 2007b
C. Trypanorhyncha *Pseudolacistorhynchus nanus* Beveridge & Justine, 2007b
Copepoda *Alebion gracilis* Boxshall & Huys, 2007

ACTINOPTERYGII
(alphabetical order of families and species; 48 families recorded with parasites; 163 species recorded with parasites)

ACANTHURIDAE
***Acanthurus blochii* Valenciennes**
Digenea *Parvipyrum acanthuri* Bray & Justine, 2008a

***Acanthurus xanthopterus* Valenciennes**
Turbellaria, unidentified Justine et al., 2009b

***Naso* sp. “unicorn fish”**
Digenea *Hapladena tanyorchis* Durio & Manter, 1969
Digenea *Quadrifoliovarium pritchardae* Manter, 1969

***Naso unicornis* (Forsskål)**
Turbellaria *Piscinquilinus* sp. Justine et al., 2009b
Monopisthocotylea *Dioncopseudobenedenia kala* Perkins et al., 2009

Nematoda Capillariidae Gen. sp. Moravec, 2001
Copepoda *Alanlewisia fallolunulus* Boxshall, 2008

***Zebrasoma veliferum* (Bloch)**
Digenea *Affecauda salacia* Hall & Cribb, 2004b

***Zebrasoma scopas* (Cuvier)**
Nematoda *Procamallanus (Spirocamallanus) longus* Moravec et al., 2006a

ACROPOMATIDAE
***Synagrops* sp. [Deep-sea]**
Isopoda *Aega musorstom* Bruce, 2004

ALBULIDAE
***Albula glossodonta* (Forsskål)**
Nematoda *Spinitectus (Paraspinitectus)* sp. Moravec & Justine, 2009b

ANGUILLIDAE
***Anguilla obscura* Günther [freshwater]**
Digenea *Stegodexamene anguillae* Moravec & Justine, 2007c
Nematoda *Procamallanus (Procamallanus) pacificus* Moravec et al., 2006b

***Anguilla reinhardtii* Steindachner [freshwater]**
Digenea *Stegodexamene anguillae* Moravec & Justine, 2007c
Nematoda *Procamallanus (Procamallanus) pacificus* Moravec et al., 2006b

ANTENNARIIDAE
Antennariidae sp. (recruiting larvae)
Digenea Hemiuridae (immature unidentifiable) Cribb et al., 2000

APOGONIDAE
***Apogon angustatus* (Smith & Radcliffe)? (recruiting larvae)**
Digenea Didymozoidae larva B Cribb et al., 2000

***Apogon coccineus* Rüppell (recruiting larvae)**
Digenea Didymozoidae larva A Cribb et al., 2000
Digenea Didymozoidae larva B Cribb et al., 2000
Digenea Didymozoidae larva E Cribb et al., 2000
Digenea Didymozoidae larva F Cribb et al., 2000

ATHERINIDAE
Myctophidae “pretre” (? *Atherinomorus lacunosus* (Forster)
Digenea *Bivesiculoides posterotestis* Durio & Manter, 1968a

BALISTIDAE
***Abalistes filamentosus* Matsuura & Yoshino**
Copepoda, unidentified Randall & Justine, 2008b
Isopoda Gnathiidae, unidentified larva Randall & Justine, 2008b

***Abalistes stellatus* Anonymous (ex Commerson)**
Digenea *Cableia balistidicola* Bray et al., 2009b
Nematoda Rhabdochonidae [as female *H. balista*, erroneous] Justine, 2007b, This paper
Nematoda *Huffmanela balista*, male Justine, 2007b
Copepoda *Hatschekia balistae* Boxshall & Huys, 2007
Copepoda *Metataeniacanthus vulgaris* Boxshall & Huys, 2007
Copepoda *Taeniacanthus aluteri* Boxshall & Huys, 2007

***Balistoides conspicillum* (Bloch & Schneider)**
Copepoda *Hatschekia balistae* Boxshall & Huys, 2007

***Pseudobalistes fuscus* (Bloch & Schneider)**
Monopisthocotylea Trochopodinae Gen. sp. 1 Perkins et al., 2009
Digenea Acanthocolpidae, unidentified Bray & Justine, 2007b
Digenea *Cableia balistidicola [*as *Cableia* sp.*]* Bray & Justine, 2007b
Digenea *Cableia balistidicola* Bray et al., 2009b
Digenea *Hypocreadium toombo* Bray & Justine, 2006a
Digenea Lepocreadiidae, unidentified Bray & Justine, 2007b
Digenea *Lobatocreadium exiguum* Bray & Justine, 2006a
Digenea *Pseudopycnadena tendu* Bray & Justine, 2007b
Digenea *Sphincteristomum acollum* Bray & Justine, 2007b
Digenea *Stephanostomum* sp. Bray & Justine, 2007b
Copepoda *Dissonus heronensi* Boxshall et al., 2008
Copepoda *Hatschekia balistae* Boxshall & Huys, 2007

Sufflamen fraenatum **(Latreille)**
Digenea *Hypocreadium patellare* Bray et al., 2009a

Belonidae

Strongylura leiura **(Bleeker) syn. of *Tylosurus leiurus* (Bleeker) ?**
Digenea *Dichadena obesa* Manter, 1969

Tylosurus crocodilus **(Péron & Lesueur)**
Digenea *Lecithostaphylus nitens* Bray & Justine, 2008a
Nematoda *Philometra dentigubernaculata* Moravec & Justine, 2009a
Copepoda *Lernanthropus tylosuri* Boxshall & Huys, 2007

Bleniidae

Bleniidae Gen. sp. (recruiting larvae)
Digenea Didymozoidae larva A Cribb et al., 2000
Digenea Hemiuridae (immature unidentifiable) Cribb et al., 2000

Bothidae

Bothus pantherinus **(Rüppell) (recruiting larvae)**
Digenea Didymozoidae larva B Cribb et al., 2000

Arnoglossus **sp. [Deep-sea]**
Isopoda *Elthusa arnoglossi* Trilles & Justine, 2006

Parabothus kiensis **[Deep-sea]**
Isopoda *Elthusa parabothi* Trilles & Justine, 2004

Carangidae

Carangoides fulvoguttatus **(Forsskål)**
Polyopisthocotylea *Heteromicrocotyla australiensis* Barton et al., 2009
Polyopisthocotylea *Heteromicrocotyloides megaspinosus* Barton et al., 2009
Copepoda *Caligus cordyla* Boxshall & Huys, 2007

Caranx papuensis **Alleyne & Macleay**
Digenea *Prosorhynchoides lamprelli* Bray & Justine, 2006b

Gnathanodon speciosus **(Forsskål)**
Nematoda *Metabronema magnum* Moravec & Justine, 2007b

Megalaspis cordyla **(Linnaeus)**
Copepoda *Lernanthropus corniger* Boxshall & Huys, 2007

Selar crumenophtalmus **(Bloch)**
Copepoda *Naobranchia* cf. *spinosa* Boxshall & Huys, 2007

Chaetodontidae

Heniochus acuminatus **(Linnaeus) "*Chaetodon acuminatus*"**
Digenea *Aponurus chelebesoi* Bray & Cribb, 2000a

Chaetodon auriga **Forsskål**
Monopisthocotylea *Aliatrema cribbi* Plaisance & Kritsky, 2004
Monopisthocotylea *Euryhaliotrematoides annulicirrus* Plaisance & Kritsky, 2004
Monopisthocotylea *Euryhaliotrematoides grandis* Plaisance & Kritsky, 2004
Monopisthocotylea *Euryhaliotrematoides pirulum* Plaisance & Kritsky, 2004
Monopisthocotylea *Haliotrema aurigae* Plaisance et al., 2004
Digenea *Aponurus chelebesoi* Bray & Cribb, 2000a

Chaetodon citrinellus **Cuvier**
Monopisthocotylea *Euryhaliotrematoides grandis* Plaisance & Kritsky, 2004
Monopisthocotylea *Haliotrema aurigae* Plaisance et al., 2004
Digenea *Aponurus chelebesoi* Bray & Cribb, 2000a

Chaetodon ephippium **Cuvier**
Digenea *Aponurus chelebesoi* Bray & Cribb, 2000a
Turbellaria, unidentified Justine et al., 2009b

Chaetodon flavirostris **Günther**
Digenea *Aponurus chelebesoi* Bray & Cribb, 2000a

Chaetodon lineolatus **Cuvier**
Digenea *Aponurus chelebesoi* Bray & Cribb, 2000a
Digenea *Cardicola chaetodontis* Nolan & Cribb, 2006

Chaetodon melannotus **Bloch & Schneider**
Digenea *Aponurus chelebesoi* Bray & Cribb, 2000a

Chaetodon mertensii **Cuvier**
Digenea *Aponurus chelebesoi* Bray & Cribb, 2000a

Chaetodon pelewensis **Kner**
Digenea *Aponurus chelebesoi* Bray & Cribb, 2000a

Chaetodon vagabundus **Linnaeus**
Monopisthocotylea *Aliatrema cribbi* Plaisance & Kritsky, 2004
Monopisthocotylea *Euryhaliotrematoides annulicirrus* Plaisance & Kritsky, 2004
Monopisthocotylea *Euryhaliotrematoides aspitis* Plaisance & Kritsky, 2004
Monopisthocotylea *Euryhaliotrematoides grandis* Plaisance & Kritsky, 2004
Monopisthocotylea *Haliotrema aurigae* Plaisance et al., 2004

Coradion altivelis **McCulloch**
Digenea *Aponurus chelebesoi* Bray & Cribb, 2000a

Forcipiger flavissimus **Jordan & McGregor**
Digenea *Aponurus chelebes*oi Bray & Cribb, 2000a

Heniochus acuminatus **(Linnaeus)**
Digenea *Aponurus chelebesoi* Bray & Cribb, 2000a

Heniochus chrysostomus **Cuvier**
Monopisthocotylea *Aliatrema cribbi* Plaisance & Kritsky, 2004
Monopisthocotylea *Euryhaliotrematoides microphallus* Plaisance & Kritsky, 2004
Monopisthocotylea *Haliotrema aurigae* Plaisance et al., 2004
Digenea *Aponurus chelebesoi* Bray & Cribb, 2000a

Heniochus monoceros **Cuvier**
Digenea *Aponurus chelebesoi* Bray & Cribb, 2000a

Chanidae

Chanos chanos **(Forsskål)**

Digenea *Isorchis parvus*	Durio & Manter, 1969

Chirocentridae

Chirocentrus dorab **(Forsskål)**

Copepoda *Parabrachiella appendiculata*	Boxshall & Huys, 2007

Coryphaenidae

Coryphaena hippurus **Linnaeus**

Copepoda *Caligus coryphaenae*	Boxshall & Huys, 2007

Echeneidae

Echeneis naucrates **Linnaeus**

Copepoda *Margolisius* cf. *abditus*	Boxshall & Huys, 2007

Elopidae

Elops hawaiensis **Regan**

Copepoda *Gloiopotes watsoni*	Boxshall & Huys, 2007

Gobiidae

Awaous guamensis **(Valenciennes) [freshwater]**

Nematoda *Camallanus cotti*	Moravec & Justine, 2006a

Haemulidae

Diagramma pictum **(Thunberg)**

Digenea *Holorchis castex*	Bray & Justine, 2007a
Digenea *Lasiotocus plectorhynchi*	Bray & Justine, 2007a
Digenea, unidentified	Baillon, 1991
Nematoda Camallanidae, unidentified	Baillon, 1991
Isopoda, unidentified adults	Baillon, 1991
Hirudinea, unidentified	Baillon, 1991

Plectorhinchus chaetodonoides **Lacépède**

Monopisthocotylea Capsalidae sp. 1	Perkins et al., 2009

Plectorhinchus goldmani **(Bleeker)**

Copepoda *Holorchis plectorhynchi*	Durio & Manter, 1968b

Plectorhinchus lineatus

Digenea *Anuretes justinei*	Venmathi Maran et al., 2008

Plectorhinchus sp. **("loche castex")**

Digenea *Lasiotocus longitestis*	Durio & Manter, 1968a
Digenea *Hysterorchis vitellosus*	Durio & Manter, 1968a
Digenea *Choanostoma secundum*	Durio & Manter, 1968b

Hemiramphidae

Hemiramphus **sp.**

Digenea *Tergestia clonacantha*	Durio & Manter, 1968a

Holocentridae

Sargocentron ensifer **(Jordan & Evermann)**

Digenea *Lecithochirium cirrhiti*	Moravec & Justine, 2009b

Sargocentron spiniferum **(Forsskål)**

Digenea *Lecithochirium cirrhiti*	Moravec & Justine, 2009b
Nematoda *Ascarophis (Dentiascarophis) adioryx*	Moravec & Justine, 2009b

Neoniphon sammara **(Forsskål)**

Digenea *Lecithochirium cirrhiti*	Moravec & Justine, 2009b
Nematoda *Ascarophis (Dentiascarophis) adioryx*	Moravec & Justine, 2009b

Hoplichthyidae

Hoplichthys citrinus **Gilbert [Deep-sea]**

Nematoda *Ascarophis (Similarascarophis) richeri*	Moravec & Justine, 2007a
Nematoda *Ichthyofilaria novaecaledoniensis*	Moravec & Justine, 2009a

Istiophoridae

Tetrapturus audax **(Philippi)**

Nematoda *Hysterothylacium cenaticum*	Moravec & Justine, 2005a
Copepoda *Gloiopotes watsoni*	Boxshall & Huys, 2007

'Espadon' (Swordfish)

Isopoda *Ceratothoa impressa*	Trilles, 1972

Kuhliidae

Kuhlia marginata **(Cuvier) [freshwater]**

Nematoda *Camallanus cotti* (larvae)	Moravec & Justine, 2006a

Labridae

Bodianus perditio **(Quoy & Gaimard)** [in Manter as "*Lepidaplois perditio*"]

Monopisthocotylea *Haliotrema banana*	Lim & Justine, 2007
Digenea *Intusatrium robustum*	Bray & Justine, 2006a; Durio & Manter, 1968b
Nematoda Cucullanidae, unidentified	Lim & Justine, 2007
Nematoda *Huffmanela ossicola*	Justine, 2007b; Lim & Justine, 2007
Copepoda *Dissonus excavatus*	Boxshall et al., 2008; Lim & Justine, 2007
Copepoda *Hatschekia* sp.	Lim & Justine, 2007
Copepoda *Lepeophtheirus* sp.	Lim & Justine, 2007
Copepoda *Lernanthropus* sp.	Lim & Justine, 2007
Isopoda Gnathiidae, unidentified larva	Lim & Justine, 2007

Bodianus loxozonus **(Snyder)**

Digenea *Intusatrium robustum*	Bray & Justine, 2006a
Nematoda *Huffmanela ossicola*	Justine, 2004

Cheilinus chlorourus **(Bloch)**

Turbellaria, unidentified	Justine et al., 2009b

Choerodon graphicus **(De Vis)**

Digenea *Postlepidapedon secundum*	Bray & Justine, 2006a

Hemigymnus melapterus **(Bloch)**

Isopoda Gnathiidae, unidentified larva	Grutter, 1999

Labridae sp. (recruiting larvae)

Digenea Didymozoidae larva D	Cribb et al., 2000
Digenea Didymozoidae larva G	Cribb et al., 2000

Labroides dimidiatus **(Valenciennes)**

Digenea *Rhipidocotyle* sp.	Jones et al., 2003, 2004

Thalassoma lutescens **(Lay & Bennett)**

Turbellaria, unidentified	Justine et al., 2009b

Thalassoma **sp. (recruiting larvae)**

Digenea Didymozoidae larva D	Cribb et al., 2000
Digenea Didymozoidae larva G	Cribb et al., 2000
C. Tetraphyllidea Metacestode	Cribb et al., 2000

LETHRINIDAE

***Gnathodentex aureolineatus* (Lacépède)**
Monopisthocotylea *Lamellodiscus* sp. Justine, 2007e; Justine & Briand, 2009

***Gymnocranius euanus* (Günther)**
Monopisthocotylea *Lamellodiscus parvicornis* Justine & Briand, 2009
Monopisthocotylea *Lamellodiscus magnicornis* Justine & Briand, 2009
2 species above as Monopisthocotylea *Lamellodiscus* sp. Justine, 2007e
Polyopisthocotylea Microcotylidae, unidentified Justine, 2007e

***Gymnocranius grandoculis* (Valenciennes)**
Monopisthocotylea *Lamellodiscus magnicornis* Justine & Briand, 2009
Monopisthocotylea *Lamellodiscus parvicornis* Justine & Briand, 2009
Monopisthocotylea *Lamellodiscus tubulicornis* Justine & Briand, 2009
3 species above as Monopisthocotylea *Lamellodiscus* sp. Justine, 2007e
Polyopisthocotylea Microcotylidae, unidentified Justine, 2007e
Nematoda *Huffmanela filamentosa* Justine, 2004
Nematoda *Huffmanela longa* Justine, 2007b

***Gymnocranius* sp. A of Justine & Briand, 2009**
Monopisthocotylea *Lamellodiscus magnicornis* Justine & Briand, 2009

***Gymnocranius* sp. B of Justine & Briand, 2009**
Monopisthocotylea *Lamellodiscus magnicornis* Justine & Briand, 2009
Monopisthocotylea *Lamellodiscus parvicornis* Justine & Briand, 2009
Monopisthocotylea *Lamellodiscus tubulicornis* Justine & Briand, 2009

***Lethrinus atkinsoni* Seale**
Monopisthocotylea *Calydiscoides rohdei* Justine, 2007e
Digenea *Zoogonus pagrosomi* Bray & Justine, 2008a

***Lethrinus genivittatus* Valenciennes**
Digenea *Zoogonus pagrosomi* Bray & Justine, 2008a
Nematoda *Philometra lethrini* Moravec & Justine, 2008, 2009a

***Lethrinus harak* Forsskål**
Monopisthocotylea *Calydiscoides difficilis* Rascalou & Justine, 2007
Monopisthocotylea *Calydiscoides duplicostatus* Rascalou & Justine, 2007
Monopisthocotylea *Calydiscoides terpsichore* Rascalou & Justine, 2007
Monopisthocotylea *Encotyllabe* sp. Rascalou & Justine, 2007
Monopisthocotylea *Haliotrema* spp. (3 species) Justine, 2007e; Rascalou & Justine, 2007
Digenea Didymozoidae, unidentified adult Rascalou & Justine, 2007
Digenea Opecoelidae, unidentified Rascalou & Justine, 2007
Copepoda *Hatschekia* sp. Rascalou & Justine, 2007
Isopoda Gnathiidae, unidentified larva Rascalou & Justine, 2007
Hirudinea, unidentified Rascalou & Justine, 2007

***Lethrinus lentjan* Lacépède**
Monopisthocotylea *Calydiscoides difficilis* Rascalou & Justine 2007
Monopisthocotylea *Calydiscoides duplicostatus* Rascalou & Justine, 2007
Monopisthocotylea *Haliotrema* sp. Justine, 2007e
Digenea *Orthodena tropica* Bray & Justine, 2007b

***Lethrinus miniatus* (Forster)**
Monopisthocotylea *Calydiscoides australis* Justine, 2007e
Monopisthocotylea *Calydiscoides gussevi* Justine, 2007e
Digenea *Hamacreadium mutabile* Durio & Manter, 1968b
Digenea *Holorchis plectorhynchi* Durio & Manter, 1968b
Digenea *Lecithochirium magnaporum* Manter, 1969
Nematoda *Philometra lethrini* Moravec & Justine, 2009a

***Lethrinus nebulosus* (Forsskål) ? (“bec de cane” in Manter)**
Monopisthocotylea *Calydiscoides difficilis* Rascalou & Justine, 2007
Monopisthocotylea *Calydiscoides duplicostatus* Rascalou & Justine, 2007
Monopisthocotylea *Calydiscoides terpsichore* Rascalou & Justine,, 2007
Monopisthocotylea *Encotyllabe* sp. Rascalou & Justine, 2007
Monopisthocotylea *Haliotrema* sp. Justine, 2007e; Rascalou & Justine, 2007
Polyopisthocotylea Microcotylidae, unidentified Rascalou & Justine, 2007
Digenea *Diphtherostomum tropicum* Durio & Manter, 1968a
Digenea *Hamacreadium diacopae* Durio & Manter, 1968b
Digenea Opecoelidae, unidentified Rascalou & Justine, 2007
Digenea *Orthodena tropica* Durio & Manter, 1968b
Digenea *Pseudoplagioporus interruptus* Durio & Manter, 1968b
Digenea *Pseudoplagioporus lethrini* Durio & Manter, 1968b
Copepoda *Hatschekia* sp. Rascalou & Justine, 2007

***Lethrinus obsoletus* (Forsskål)**
Monopisthocotylea *Calydiscoides difficilis* Rascalou & Justine, 2007

***Lethrinus ravus* Carpenter & Randall**
Monopisthocotylea *Calydiscoides difficilis* Rascalou & Justine, 2007
Monopisthocotylea *Haliotrema* sp. Justine, 2007e
Nematoda *Procamallanus (Spirocamallanus)* sp. 2 Moravec et al., 2006a

***Lethrinus rubrioperculatus* Sato**
Monopisthocotylea *Calydiscoides euzeti* Justine, 2007e
Monopisthocotylea *Encotyllabe* sp. Justine, 2007e
Monopisthocotylea *Haliotrema* sp. Justine, 2007e
Digenea Didymozoidae, unidentified Justine, 2007e
Digenea *Pseudoplagioporus interruptus* Bray & Justine, 2007b; Justine, 2007e

C. Trypanorhyncha *Nybelinia* sp. Justine, 2007e
C. Trypanorhyncha *Otobothrium* sp. Justine, 2007e
Copepoda *Caligus lethrinicola* Boxshall & El-Rashidy, 2009
as Copepoda *Caligus* cf. *mauritanicus* Boxshall & Huys, 2007; Justine, 2007e
Isopoda Gnathiidae, unidentified larva Justine, 2007e

Lethrinus variegatus **Valenciennes**
Nematoda *Philometra lethrini* Moravec & Justine, 2009a

Lethrinus xanthochilus **Klunzinger**
Monopisthocotylea *Calydiscoides euzeti* Justine, 2007e
Monopisthocotylea *Encotyllabe* sp. Justine, 2007e
Monopisthocotylea *Haliotrema* sp. Justine, 2007e
Copepoda, unidentified larvae Justine, 2007e

LUTJANIDAE

Aprion virescens **Valenciennes**
Monopisthocotylea *Pseudonitzschia uku* Perkins et al., 2009
Nematoda *Cucullanus bourdini* Petter & Le Bel, 1992

Etelis carbunculus **Cuvier [Deep-sea]**
Digenea *Neolebouria blatta* Bray & Justine, 2009a
Isopoda *Anilocra gigantea* Trilles, 1972

'Lutjanus amabilis' **(??? not in FishBase)**
Digenea *Hamacreadium mutabile* Durio & Manter, 1968b
Isopoda *Ceratothoa carinata* Trilles, 1972

Lutjanus argentimaculatus **(Forsskål)**
Monopisthocotylea *Euryhaliotrema* sp. Kritsky et al., 2009
Monopisthocotylea *Haliotrematoides novaecaledoniae* Kritsky et al., 2009
Monopisthocotylea *Haliotrematoides potens* Kritsky et al., 2009
Digenea *Stephanostomum casum* Durio & Manter, 1969

Lutjanus bohar **(Forsskål) ? ("anglais" in Manter)**
Digenea *Hamacreadium mutabile* Durio & Manter, 1968b
Digenea *Siphoderina catalae* Durio & Manter, 1969
Digenea *Siphoderina longitestis* Durio & Manter, 1969
Digenea *Siphoderina paracatalae* Durio & Manter, 1969
Digenea *Siphoderina testitactus* Durio & Manter, 1969

Lutjanus fulviflamma **(Forsskål)**
Monopisthocotylea *Euryhaliotrema* sp. Kritsky et al., 2009
Monopisthocotylea *Haliotrematoides patellacirrus* Kritsky et al., 2009
Monopisthocotylea *Haliotrematoides tainophallus* Kritsky et al., 2009
Digenea *Hamacreadium mutabile* Bray & Justine, 2007b
Digenea *Lobosorchis tibaldiae* Miller & Cribb, 2005
Digenea *Retrovarium* sp. Miller & Cribb, 2007

Lutjanus fulvus **(Forster) (as *Lutjanus vaigiensis* in Manter, junior synonym)**
Monopisthocotylea Ancyrocephalidae, unidentified Kritsky et al., 2009
Monopisthocotylea *Euryhaliotrema* sp. Kritsky et al., 2009
Monopisthocotylea *Haliotrematoides longitubocirrus* Kritsky et al., 2009
Monopisthocotylea *Haliotrematoides patellacirrus* Kritsky et al., 2009
Digenea *Hexangium sigani* Durio & Manter, 1968a
Digenea *Lecithochirium polynemi* Manter, 1969
Digenea *Siphoderina provitellosus* Durio & Manter, 1968b

Lutjanus kasmira **(Forsskål)**
Monopisthocotylea Ancyrocephalidae, unidentified Kritsky et al., 2009
Digenea *Hamacreadium mutabile* Bray & Justine, 2007b

Lutjanus quinquelineatus **(Bloch)**
Monopisthocotylea Ancyrocephalidae, unidentified Kritsky et al., 2009
Monopisthocotylea *Euryhaliotrema* sp. Kritsky et al., 2009
Monopisthocotylea *Haliotrematoides lanx* Kritsky et al., 2009
Monopisthocotylea *Haliotrematoides longitubocirrus* Kritsky et al., 2009
Monopisthocotylea *Haliotrematoides patellacirrus* Kritsky et al., 2009

Lutjanus russellii **(Bleeker)**
Monopisthocotylea *Euryhaliotrema* sp. Kritsky et al., 2009
Monopisthocotylea *Haliotrematoides longitubocirrus* Kritsky et al., 2009
Monopisthocotylea *Haliotrematoides patellacirrus* Kritsky et al., 2009

Lutjanus vitta **(Quoy & Gaimard)**
Monopisthocotylea Ancyrocephalidae, unidentified Kritsky et al., 2009
Monopisthocotylea *Euryhaliotrema* sp. Kritsky et al., 2009
Monopisthocotylea *Haliotrematoides longitubocirrus* Kritsky et al., 2009
Monopisthocotylea *Haliotrematoides patellacirrus* Kritsky et al., 2009
Digenea *Choanostoma secundum* Durio & Manter, 1968b
Nematoda *Philometra* sp. Moravec & Justine, 2008

Macolor niger **(Forsskål)**
Copepoda *Dissonus excavatus* Boxshall et al., 2008

Pristipomoides argyrogrammicus **(Valenciennes) [Deep-sea]**
Digenea *Neolebouria blatta* Bray & Justine, 2009a
Copepoda *Lernaeolophus striatus* Boxshall & Huys, 2007

Pristipomoides filamentosus **(Valenciennes) [Deep-sea]**
Nematoda *Cucullanus bourdini* Petter & Le Bel, 1992

Pristipomoides flavipinnis **Shinohara [Deep-sea]**
Nematoda *Cucullanus bourdini* Petter & Le Bel, 1992

MACROURIDAE

Hymenocephalus gracilis **Gilbert & Hubbs [Deep-sea]**
Copepoda *Sarcotretes scopeli* Boxshall, 1989

MONACANTHIDAE

Aluterus monoceros **(Linnaeus)**
Digenea *Aponurus laguncula* Quilichini et al., 2009b

MUGILIDAE

Mugilidae
Digenea *Hymenocotta mulli* Durio & Manter, 1968a

MULLIDAE

Mulloidichthys vanicolensis **(Valenciennes)**
Monopisthocotylea *Pennulituba cymansis* Řehulková et al., 2009

Monopisthocotylea *Pennulituba piratifalx* Řehulková et al., 2009
Monopisthocotylea *Volsellituba elephantina* Řehulková et al., 2009
Monopisthocotylea *Volsellituba nabla* Řehulková et al., 2009
Monopisthocotylea *Volsellituba orchidea* Řehulková et al., 2009
Digenea *Homalometron moraveci* Bray et al., 2007

***Parupeneus multifasciatus* (Quoy & Gaimard)**
Digenea *Deretrema combesae* Bray & Justine, 2008b
Digenea *Deretrema combesorum* Bray & Justine, 2008b
Digenea Didymozoidae, unidentified juveniles Bray & Justine, 2008b
Digenea *Lecithochirium* sp. Bray & Justine, 2008b
Digenea *Phyllodistomum* sp. Bray & Justine, 2008b

***Parupeneus ciliatus* (Lacépède)**
Nematoda *Camallanus carangis* Moravec et al., 2006a

***Upeneus vittatus* (Forsskål)**
Nematoda *Camallanus carangis* Moravec et al., 2006a
Copepoda *Irodes sauridi* Boxshall & Huys, 2007

NEMIPTERIDAE
***Nemipterus furcosus* (Valenciennes)**
Turbellaria, unidentified Justine et al., 2009b
Monopisthocotylea *Benedenia* sp. Justine & Brena, 2009
Polyopisthocotylea Microcotylidae, unidentified Justine & Brena, 2009
Digenea *Macvicaria jagannathi* Bray & Justine, 2009b
Digenea *Neolebouria lineatus* Bray & Justine, 2009b
Nematoda *Camallanus carangis* Moravec et al., 2006a
Nematoda *Huffmanela branchialis* Justine, 2004
Nematoda *Raphidascaris (Ichtyiascaris) nemipteri* Moravec & Justine, 2005a

***Pentapodus aureofasciatus* Russell**
[*Pentapodus* sp. in Justine, 2004]
Monopisthocotylea *Calydiscoides limae* Justine & Brena, 2009
Polyopisthocotylea Microcotylidae, unidentified Justine & Brena, 2009
Nematoda *Huffmanela* sp. Justine, 2004, 2007b
Isopoda Gnathiidae, unidentified larva Justine & Brena, 2009

***Scolopsis bilineata* (Bloch)**
Nematoda *Procamallanus (Spirocamallanus)* sp. 2 Moravec et al., 2006a
Isopoda Gnathiidae, unidentified larva Grutter, 1999

PINGUIPEDIDAE
***Parapercis hexophtalma* (Cuvier)**
Nematoda *Procamallanus (Spirocamallanus)* sp. 1 Moravec et al., 2006a

POMACANTHIDAE
***Pomacanthus semicirculatus* (Cuvier)**
Copepoda *Pseudanuretes fortipedis* Boxshall & Huys, 2007

POMACENTRIDAE
***Chromis viridis* (Cuvier)**
Turbellaria, unidentified Justine et al., 2009b

PRIACANTHIDAE
***Priacanthus hamrur* (Forsskål)**
Digenea *Dupliciporia lanterna* Bray & Justine, 2008a
Digenea *Parahemiurus merus* Bray & Cribb, 2005
Nematoda *Philometra priacanthi* Moravec & Justine, 2009a
Copepoda *Norion priacanthi* Boxshall & Huys, 2007

SCARIDAE
Scaridae? "Brown-blotched parrot fish" [Probably erroneous for Labridae]
Digenea *Intusatrium secundum* Durio & Manter, 1968b

***Scarus* (= *Callyodon*) sp.**
Digenea *Lecithaster testilobatus* Manter, 1969

SCOMBRIDAE
***Acanthocybium solandri* (Cuvier)**
Polyopisthocotylea *Neothoracocotyle acanthocybii* Rohde et al., 1980
Copepoda *Brachiella thynni* Rohde et al., 1980
Copepoda *Caligus productus* Rohde et al., 1980
Copepoda *Gloiopotes hygomianus* Boxshall & Huys, 2007

***Katsuwonus pelamis* (Linnaeus)**
Polyopisthocotylea *Allopseudaxine* sp. Rohde et al., 1980
Polyopisthocotylea *Allopseudaxinoides vagans* Rohde et al., 1980
Digenea *Syncoelium filiferum* Rohde et al., 1980
Nematoda *Ctenascarophis lesteri* Crites et al., 1993
Copepoda *Caligus bonito* Rohde et al., 1980
Copepoda *Caligus productus* Rohde et al., 1980

***Rastrelliger kanagurta* (Cuvier)**
Copepoda *Pumiliopes capitulatus* Boxshall & Huys, 2007

***Scomberomorus commerson* (Lacépède)**
Copepoda *Caligus infestans* Boxshall & Huys, 2007

***Thunnus albacares* (Bonnaterre)**
Copepoda *Caligus bonito* Boxshall & Huys, 2007
Copepoda *Pseudocycnus appendiculatus* Boxshall & Huys, 2007
Scombridae "mackerel"
Digenea *Lecithocladium aegyptensis* Manter, 1969

SCORPAENIDAE
***Dendrochirus zebra* (Cuvier)**
C. Trypanorhyncha *Nybelinia syngenes* Palm, 2004; Palm & Beveridge, 2002

Scorpaenidae Gen. sp. (recruiting larvae)
Digenea Didymozoidae larva A Cribb et al., 2000

SERRANIDAE
***Cephalopholis argus* Bloch & Schneider**
Monopisthocotylea *Pseudorhabdosynochus argus* Justine, 2007d
Isopoda Gnathiidae, unidentified larva Justine, 2007d

***Cephalopholis aurantia* × *C. spiloparaea* (Hybrid, cf. Randall & Justine, 2008a)**
C. Bothriocephalidea *Bothriocephalus celineae* Kuchta et al., 2009a

C. Bothriocephalidea *B. celineae* [as pseudophyll. cestode]	Randall & Justine, 2008a
Copepoda, unidentified larvae	Randall & Justine, 2008a

***Cephalopholis boenak* Bloch**

Isopoda Gnathiidae, unidentified larva	Justine, 2007d

***Cephalopholis sonnerati* (Valenciennes)**

Monopisthocotylea *Pseudorhabdosynochus minutus*	Justine, 2007d
Monopisthocotylea *Diplectanum nanus*	Justine, 2007d
Monopisthocotylea *Haliotrema* sp.	Justine, 2007d
Copepoda, unidentified larva	Justine, 2007d
Isopoda Gnathiidae, unidentified larva	Justine, 2007d

***Cephalopholis urodeta* (Forster)**

Monopisthocotylea *Diplectanum parvus*	Justine, 2008a
Digenea, unidentified	Justine, 2008a
C. Tetraphyllidea larvae, unidentified	Justine, 2008a
C. Trypanorhyncha *Floriceps minacanthus*	Justine, 2008a
Nematoda Anisakidae larvae, unidentified	Justine, 2008a
Copepoda, unidentified	Justine, 2008a
Isopoda Gnathiidae, unidentified larva	Justine, 2008a

***Epinephelus areolatus* (Forsskål)**

Nematoda *Philometra ocularis*	Moravec & Justine, 2008, 2009a

***Epinephelus chlorostigma* (Valenciennes) [Deep-sea]**

Monopisthocotylea *Pseudorhabdosynochus epinepheli*	Justine, 2009b
Copepoda *Sagum epinepheli*	Boxshall & Huys, 2007

***Epinephelus coioides* (Hamilton)**

C. Trypanorhyncha *Pseudogilquinia pillersi*	Beveridge et al., 2007
Nematoda *Philometra ocularis*	Moravec & Justine, 2005b

***Epinephelus coeruleopunctatus* (Bloch)**

Monopisthocotylea *Haliotrema* sp.	Sigura et al., 2007
Monopisthocotylea *Pseudorhabdosynochus bacchus*	Sigura et al., 2007
Monopisthocotylea *Pseudorhabdosynochus* spp.	Sigura et al., 2007
Digenea *Cainocreadium epinepheli*	Bray & Justine, 2007b
Copepoda *Sagum epinepheli*	Boxshall & Huys, 2007
as Copepoda, unidentified	Sigura et al., 2007

***Epinephelus cyanopodus* (Richardson)**

Monopisthocotylea *Allobenedenia* sp.	Sigura & Justine, 2008
Monopisthocotylea *Haliotrema* sp.	Sigura & Justine, 2008
Monopisthocotylea *Laticola cyanus*	Sigura & Justine, 2008
Monopisthocotylea *Pseudorhabdosynochus chauveti*	Sigura & Justine, 2008
Monopisthocotylea *Pseudorhabdosynochus cyanopodus*	Sigura & Justine, 2008
Monopisthocotylea *Pseudorhabdosynochus duitoe*	Sigura & Justine, 2008
Monopisthocotylea *Pseudorhabdosynochus exoticus*	Sigura & Justine 2008
Monopisthocotylea *Pseudorhabdosynochus huitoe*	Sigura & Justine, 2008
Monopisthocotylea *Pseudorhabdosynochus podocyanus*	Sigura & Justine, 2008
Digenea *Allopodocotyle epinepheli*	Bray & Justine, 2007b
Digenea *Cainocreadium epinepheli*	Sigura & Justine, 2008
Digenea Didymozoidae unidentified sp.1	Sigura & Justine, 2008
Digenea Didymozoidae unidentified sp.2	Sigura & Justine, 2008
Digenea *Erilepturus hamati*	Manter, 1969; Sigura & Justine, 2008
C. Tetraphyllidea, unidentified larvae	Sigura & Justine, 2008
C. Trypanorhyncha *Floriceps minacanthus*	Sigura & Justine, 2008
C. Trypanorhyncha *Pseudolacistorhynchus heroniensis*	Sigura & Justine, 2008
Nematoda *Philometra cyanopodi [as Ph. lateolabracis]*	Moravec & Justine, 2005b
Nematoda *Philometra cyanopodi*	Moravec & Justine, 2008
Nematoda *Philometra ocularis*	Moravec & Justine, 2005b
Nematoda *Procamallanus* sp.	Sigura & Justine, 2008
Nematoda *Terranova* sp., larvae	Sigura & Justine, 2008
Copepoda *Dissonus manteri*	Boxshall et al., 2008)
Copepoda *Hatschekia cernae*	Sigura & Justine, 2008
Copepoda *Hatschekia* sp. (2 species)	Sigura & Justine, 2008
Copepoda Pennellidae, unidentified larvae	Sigura & Justine, 2008
Copepoda *Sagum epinepheli*	Boxshall & Huys, 2007; Sigura & Justine, 2008
Isopoda Gnathiidae, unidentified larva	Sigura & Justine, 2008
Isopoda *Argathona rhinoceros*	Sigura & Justine, 2008

***Epinephelus fasciatus* (Forsskål)**

Turbellaria, unidentified	Justine et al., 2009b
Monopisthocotylea *Benedenia* cf. *epinepheli*	Justine, 2005b
Monopisthocotylea *Pseudorhabdosynochus caledonicus*	Hinsinger & Justine, 2006a; Justine, 2005b
Monopisthocotylea *Pseudorhabdosynochus youngi*	Justine et al., 2009a
Monopisthocotylea *Ps. youngi [*as *Ps. cupatus]*	Hinsinger & Justine, 2006a; Justine, 2005b
Digenea *A. novaecaledoniae* [as *Siphoderina elongata*]	Quilichini et al., 2009a
Digenea *Adlardia novaecaledoniae*	Miller et al., 2009
Digenea *Allopodocotyle epinepheli*	Bray & Justine, 2007b
Digenea *Cainocreadium epinepheli*	Bray & Justine, 2007b
Digenea *Helicometra epinepheli*	Bray & Justine, 2007b
Digenea *Lepidapedoides angustus*	Bray & Justine, 2006a
Nematoda *Philometra fasciati* [as *Ph. lateolabracis*]	Moravec & Justine, 2005b
Nematoda *Philometra fasciati*	Moravec & Justine, 2008
Nematoda *Procamallanus (Spirocamallanus) variolae*	Moravec et al., 2006a
Copepoda *Hatschekia* sp.	Justine, 2005b

***Epinephelus fuscoguttatus* (Forsskål)**

Monopisthocotylea Capsalidae Trochopodinae Gen. sp. 4	Perkins et al., 2009

***Epinephelus howlandi* (Günther)**

Monopisthocotylea *Pseudorhabdosynochus venus*	Hinsinger & Justine, 2006b
Monopisthocotylea *Pseudorhabdosynochus cyathus*	Hinsinger & Justine, 2006a

***Epinephelus maculatus* (Bloch)**

Monopisthocotylea *Diplectanum uitoe* Justine, 2007c
Monopisthocotylea *Haliotrema epinepheli* Justine, 2007c
Monopisthocotylea *Haliotrema* sp. Justine, 2007c
Monopisthocotylea *Laticola dae* Journo & Justine, 2006
Monopisthocotylea *Pseudorhabdosynochus auitoe* Justine, 2007c
Monopisthocotylea *Pseudorhabdosynochus buitoe* Justine, 2007c
Monopisthocotylea *Pseudorhabdosynochus cuitoe* Justine, 2007c
Monopisthocotylea *Pseudorhabdosynochus duitoe* Justine, 2007c
Monopisthocotylea *Pseudorhabdosynochus euitoe* Justine, 2007c
Monopisthocotylea *Pseudorhabdosynochus fuitoe* Justine, 2007c
Monopisthocotylea *Pseudorhabdosynochus guitoe* Justine, 2007c
Monopisthocotylea *Pseudorhabdosynochus huitoe* Justine, 2007c
Digenea Didymozoidae, unidentified adult Journo & Justine, 2006
Copepoda *Dissonus manteri* Boxshall et al., 2008; Journo & Justine, 2006
Copepoda *Hatschekia* sp. (2 species) Journo & Justine, 2006
Isopoda Gnathiidae, unidentified larva Journo & Justine, 2006

***Epinephelus malabaricus* (Bloch & Schneider)**

Monopisthocotylea *Diplectanum maa* Justine & Sigura, 2007
Monopisthocotylea *Pseudorhabdosynochus* cf. *shenzhenensis* Justine & Sigura, 2007
Monopisthocotylea *Pseudorhabdosynochus maaensis* Justine & Sigura, 2007
Monopisthocotylea *Pseudorhabdosynochus malabaricus* Justine & Sigura, 2007
Monopisthocotylea *Pseudorhabdosynochus manifestus* Justine & Sigura, 2007
Monopisthocotylea *Pseudorhabdosynochus manipulus* Justine & Sigura, 2007
Monopisthocotylea *Pseudorhabdosynochus marcellus* Justine & Sigura, 2007
Monopisthocotylea *Pseudorhabdosynochus maternus* Justine & Sigura, 2007
Digenea *Cainocreadium* sp. Justine & Sigura, 2007
Digenea *Prosorhynchus maternus* Bray & Justine, 2006b
C. Trypanorhyncha *Pseudogilquinia pillersi* Beveridge et al., 2007
C. Trypanorhyncha *Pseudotobothrium dipsacum* Justine & Sigura, 2007
Copepoda *Hatschekia* sp. Justine & Sigura, 2007

***Epinephelus merra* (Bloch)**

Monopisthocotylea *Benedenia* sp. Hinsinger & Justine, 2006a
Monopisthocotylea *Pseudorhabdosynochus* cf. *coioidesis* Hinsinger & Justine, 2006a
Monopisthocotylea *Pseudorhabdosynochus melanesiensis* Hinsinger & Justine, 2006a; Justine, 2005b
Digenea *Allopodocotyle epinepheli* Bray & Justine, 2007b
Digenea *Helicometra epinepheli* Bray & Justine, 2007b
Digenea *Helicometra fasciata* Rigby et al., 1997
Digenea *Lepidapedoides angustus* Rigby et al., 1997
C. Tetraphyllidea '*Scolex polymorphus*' Rigby et al., 1997
C. Trypanorhyncha larvae Rigby et al., 1997
Copepoda *Aethon* sp. Boxshall & Huys, 2007
Copepoda *Sagum epinepheli* Boxshall & Huys, 2007

***Epinephelus morrhua* (Valenciennes) [Deep-sea]**

Isopoda Gnathiidae, unidentified larva Justine, 2008c
Monopisthocotylea *Haliotrema* sp. Justine, 2008c
Monopisthocotylea *Pseudorhabdosynochus morrhua* Justine, 2008c
Monopisthocotylea *Pseudorhabdosynochus variabilis* Justine, 2008c
C. Trypanorhyncha, unidentified larva Justine, 2008c
Copepoda *Sagum epinepheli* Boxshall & Huys, 2007

***Epinephelus rivulatus* (Valenciennes)**

Monopisthocotylea *Pseudorhabdosynochus calathus* Hinsinger & Justine, 2006a
Monopisthocotylea *Pseudorhabdosynochus inversus* Justine, 2008b
Nematoda *Philometra ocularis* Moravec & Justine, 2005b
Isopoda Gnathiidae, unidentified larva Hinsinger & Justine, 2006a

***Epinephelus* sp.**

Digenea *Neidhartia coronata* Durio & Manter, 1968a
Digenea *Prosorhynchus freitasi* Durio & Manter, 1968a
Digenea *Pacificreadium serrani* Durio & Manter 1968b
Digenea *Lecithochirium magnaporum* Manter, 1969
Digenea *Erilepturus tiegsi* Manter, 1969

***Epinephelus* sp. "Red Cod"**

Digenea *Helicometra fasciata* Durio & Manter, 1968b
Digenea *Lepidapedoides dollfusi* (as *Neolepidapedon dollfusi*) Durio & Manter, 1968b
Digenea *Stephanostomum japonocasum* Durio & Manter 1969

***Epinephelus* sp. "Spotted Grouper"**

Digenea *Lepidapedoides dollfusi* (as *Neolepidapedon dollfusi*) Durio & Manter 1968b

***Grammistes* sp.**

Isopoda *Nerocila excisa* Trilles, 1972

***Plectropomus laevis* (Lacépède)**

Monopisthocotylea *Echinoplectanum chauvetorum* Justine & Euzet, 2006
Monopisthocotylea *Echinoplectanum laeve* Justine & Euzet, 2006
C. Trypanorhyncha *Pseudogilquinia pillersi* Beveridge et al., 2007
Copepoda *Dissonus manteri* Boxshall et al., 2008
Copepoda *Hatschekia* cf. *plectropomi* Boxshall & Huys, 2007
Isopoda *Argathona macronema* Justine & Euzet, 2006

***Plectropomus leopardus* (Lacépède)**

Monopisthocotylea Capsalidae Trochopodinae Gen. sp. Justine & Euzet, 2006; Perkins et al., 2009
Monopisthocotylea *Echinoplectanum leopardi* Justine & Euzet, 2006
Monopisthocotylea *Echinoplectanum pudicum* Justine & Euzet, 2006
Monopisthocotylea *Echinoplectanum rarum* Justine & Euzet, 2006

Digenea *Pacificreadium serrani* Bray & Justine, 2007b
Copepoda *Dissonus manteri* Boxshall et al., 2008
Copepoda *Hatschekia* cf. *plectropomi* Boxshall & Huys, 2007
Copepoda *Sagum epinepheli* Boxshall & Huys, 2007
Isopoda *Argathona rhinoceros* Justine & Euzet, 2006

***Pseudogramma* sp. (recruiting larvae)**
Digenea Didymozoidae larva A Cribb et al., 2000
Digenea Hemiuridae *Ectenurus* sp. adult Cribb et al., 2000

Serranidae (“leche”/ loche) several species?
Digenea *Allopodocotyle serrani* Durio & Manter, 1968b
Digenea *Myorhynchus pritchardae* Durio & Manter, 1968a
Digenea *Prosorhynchus longisaccatus* Durio & Manter, 1968a

Serranidae “unidentified serranid”
Digenea *Stephanostomum japonocasum* Durio & Manter, 1969

Serranidae “mottled grouper”
Digenea *Erilepturus tiegsi* Manter, 1969

***Variola albimarginata* Baissac**
Monopisthocotylea *Haliotrema epinepheli* Justine, 2005c
Nematoda *Procamallanus (Spirocamallanus) variolae* Moravec et al., 2006a

***Variola louti* (Forsskål)**
Monopisthocotylea *Haliotrema epinepheli* Justine, 2005c
Monopisthocotylea *Pseudorhabdosynochus hirundineus* Justine, 2005c
Digenea *Cainocreadium epinepheli* Bray & Justine, 2007b
Digenea *Prosorhynchus serrani* Durio & Manter, 1968a
Nematoda *Philometra ocularis* Moravec & Justine, 2005b

Siganidae

***Siganus argenteus* (Quoy & Gaimard)**
Digenea *Ptychogyliauchen thistilbardi* Hall & Cribb, 2004a

***Siganus canaliculatus* (Park)**
as *Siganus oramin*
Monopisthocotylea *Tetrancistrum sigani*
(as *T. nebulosi)* Young, 1967a
Digenea *Ptychogyliauchen thistilbardi* Hall & Cribb, 2004a

***Siganus corallinus* (Valenciennes)**
Digenea *Ptychogyliauchen thistilbardi* Hall & Cribb, 2004a

***Siganus doliatus* Guérin-Méneville**
Digenea *Hysterolecithoides frontilatus* Bray & Cribb, 2000b
Digenea *Ptychogyliauchen thistilbardi* Hall & Cribb, 2004a
Nematoda Capillariidae Gen. sp. Moravec, 2001

Siganus fuscescens* (Houttuyn) as *Siganus nebulosus
Monopisthocotylea *Tetrancistrum sigani*
(as *T. nebulosi)* Young, 1967a

***Siganus spinus* (Linnaeus)**
Digenea *Ptychogyliauchen thistilbardi* Hall & Cribb, 2004a

***Siganus* sp.**
Monopisthocotylea *Tetrancistrum sigani*
(as *T. nebulosi)* Young, 1967a
Digenea *Atractotrema sigani* Durio & Manter, 1969
Digenea *Gyliauchen papillatus* Durio & Manter, 1969
Digenea *Hexangium sigani* Durio & Manter, 1968a
Digenea *Hysterolecitha sigani* Manter, 1969
Digenea *Paracryptogonimus saccatus* Durio & Manter,1969
Digenea *Theletrum frontilatum* Manter, 1969

Sillaginidae

***Sillago sihama* (Forsskål)**
Polyopisthocotylea *Polylabris sillaginae* Hayward, 1996

Sphyraenidae

***Sphyraena barracuda* (Edwards)**
Copepoda *Caligus lobodes* Boxshall & Huys, 2007

***Sphyraena forsteri* Cuvier**
Copepoda *Hatschekia sphyraeni* Boxshall & Huys, 2007

***Sphyraena* sp. (recruiting larvae)**
Monopisthocotylea Capsalidae larva Cribb et al., 2000

Synaphobranchidae

***Synaphobranchus* cf. *brevidorsalis* Günther [Deep-sea]**
Copepoda *Laminohatschekia synaphobranchi* Boxshall, 1989
Copepoda *Lophoura cornuta* Boxshall, 1989

Synodontidae

***Saurida nebulosa* Valenciennes**
C. Bothriocephalidea *Oncodiscus sauridae* Kuchta et al., 2009b

***Saurida undosquamis* (Richardson)**
C. Bothriocephalidea *Oncodiscus sauridae* Kuchta et al., 2009b

***Synodus similis* McCulloch**
Copepoda *Metataeniacanthus vulgaris* Boxshall & Huys, 2007

***Synodus variegatus* (Lacépède)**
Copepoda *Metataeniacanthus vulgaris* Boxshall & Huys, 2007

Synodontidae Gen. sp. (recruiting larvae)
Digenea Didymozoidae larva A Cribb et al., 2000

Synodontidae Gen. sp. 1 (recruiting larvae)
Digenea Didymozoidae larva C Cribb et al., 2000
Digenea *Erilepturus* sp. immature Cribb et al., 2000

Synodontidae Gen. sp. 2 (recruiting larvae)
Digenea *Ectenurus* sp. immature Cribb et al., 2000

Synodontidae Gen. sp. 3 (recruiting larvae)
Cestoda metacestode Cribb et al., 2000

Tetraodontidae

***Lagocephalus sceleratus* (Gmelin)**
Nematoda *Philometra lagocephali* Moravec & Justine, 2008
Nematoda *Philometra tenuicauda* Moravec & Justine, 2009a
Copepoda *Taeniacanthus kitamakura* Boxshall & Huys, 2007

Trichiuridae

***Assurger anzac* (Alexander)**
Copepoda *Avitocaligus assurgericola* Boxshall & Justine, 2005

TABLE 3

Species of fish parasites recorded from New Caledonia. From the parasite-host list in Table 1. The total of Platyhelminthes species is 240 (65% of the total).

Group	Families	Species
Turbellaria	1	1
Monopisthocotylea	4	98 (27%)
Polyopisthocotylea	5	8
Digenea	20	105 (29%)
Cestoda Bothriocephalidea	1	2
Cestoda Tetraphyllidea	1	6
Cestoda Trypanorhyncha	5	20
Nematoda	10	41 (11%)
Copepoda	13	61 (16%)
Isopoda	4	27 (7%)
Acanthocephala	1	1
Hirudinea	1	1
Total		371

TABLE 4

Biodiversity of parasites in three species of groupers (Family Serranidae). First number is the number of species recorded in New Caledonia; second number in parenthesis is the total number of species recorded in all locations. From Justine & Sigura (2007). 'Others' include Hirudinea and Acanthocephala.

	Monogenea	Digenea	Cestoda	Nematoda	Copepoda	Isopoda	Others	Total
Epinephelus maculatus	12 (12)	7 (7)	1 (2)	1 (1)	3 (3)	1 (1)	0 (0)	25 (26)
Epinephelus cyanopodus	6 (7)	4 (10)	2 (2)	4 (8)	5 (5)	2 (2)	0 (0)	23 (34)
Epinephelus malabaricus	11 (15)	2 (11)	2 (3)	1 (4)	2 (8)	0 (1)	0 (2)	18 (44)

TABLE 5

Available references on fish parasites in the Indo-Pacific Region. Some studies may include freshwater fish and their parasites. Protozoa are included in all studies except the present one.

Reference	Parasite groups	Locality	Number of fish species with parasites	Number of fish species present	Number of parasites
Arthur & Te, 2006	all	Viet Nam	130	1300 *	453
Arthur & Lumanlan Mayo, 1997	all	Philippines	172	2030 *	201
Arthur & Ahmed, 2002	all	Bangladesh	85	528 *	147
Beumer et al., 1983	all	Australia (including Antarctic territories)	448	? *	496
Lester & Sewell, 1989	all	Heron Island, Australia	122	ca 2,000	ca 300 species ** 580 records
This study	**Metazoa**	**New Caledonia**	**195**	**ca 2,000**	**371 species** **580 records**

* include freshwater fishes ** difficult to evaluate since many identifications are at the family level only.

Includes ca 140 species of monogeneans.

TABLE 6

Numerical evaluations of fish parasite biodiversity in the literature.

Parasite groups	Number of parasite per fish species	Total number of parasites	Comment	Reference
Monogeneans	3	882	Marine fish, Peninsular Malaysia	Lim, 1998
Monogeneans	5		Estimate, freshwater fishes, neotropics	Kritsky in Whittington, 1998
Monogeneans	1	25,000 species in the World	considered an underestimate by author	Whittington, 1998
Digeneans	2.73	3,800 in 2,000 fish species	Great Barrier Reef, Australia	Cribb & Bray, 1998
All Metazoa	3.1		Marine fish, German coastal waters	Palm & Klimpel, 2006
All Metazoa	5.4		Marine fish, Canada	Poulin, 2004
All Metazoa	3.0		Marine fish, New Zealand	Poulin, 2004
All, including Protozoa	20 *	20,000 for 1,000 fish species	Marine fish, Heron Island, Queensland, Australia	Rohde, 1976

* not expressly indicated in the text; calculated.

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ABSTRACTS

When names are wrong and colours deceive: unravelling the *Pseudoceros bicolor* species complex (Polycladida)

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KEY WORDS: *Pseudoceros bicolor marcusorum* n. subsp., *Maritigrella aureolineata* comb.nov., Caribbean, colour morphs.

The polyclad *Pseudoceros bicolor* Verrill, 1901 derives its name from a distinctive two-coloured dorsal colouration, which according to the original species description, consists of a very dark, almost black central area with acute black lobes extending towards a translucent grayish margin (1). At least three different colour morphs have been described, ranging from a pale speckled brown through an evenly dark brown form, to a morphotype resembling the original species description (1-9). *Pseudoceros aureolineatus* (Verrill, 1901) is easily confused with the light brown colour morph of *P. bicolor,* and *P. rawlinsonae* Bolaños, Quiroga & Litvaitis, 2007 resembles both *P. bicolor* and *P. aureolineatus.* Using morphological characters of newly collected specimens and nucleotide sequences of the D1-D2 expansion segment (28S rDNA) the taxonomic validity of *P. rawlinsonae* and *P. bicolor* is confirmed and the description of their colourations emended. At the same time, the new sub-species, *P. bicolor marcusorum* nov. subsp. is erected, the genus *Cryptoceros* Faubel, 1984 is eliminated, and the new combination *Maritigrella aureolineata* nov. comb. (Euryleptidae) is established for *P. aureolineatus.*

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First report of two monogenean parasites (*Calydiscoides* sp. and *Encotyllabe* sp.) from *Lethrinus nebulosus* in the Persian Gulf.

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The family Lethrinidae is present in the north of the Persian Gulf and consists of four species, all members of the genus *Lethrinus* Cuvier, 1829. In this study, we performed a survey of monogenean fauna of *Lethrinus nebulosus* (Forsskål, 1775), locally called she'ri, a commercial marine fish species in south of Iran. Specimens of *L. nebulosus* were caught with trawl net, then taken to the laboratory. Seventy fish were measured and weighed. Gills were extracted and frozen for future examination.

The monogeneans *Calydiscoides* sp. (Diplectanidae) and *Encotyllabe* sp. (Capsalidae) are here reported from *L. nebulosus*.

Members of *Calydiscoides* Young, 1969 are characterized by two lamellodiscs with concentric lamellae which telescope into each other, and are parasites exclusively in fishes of the family Lethrinidae and Nemipteridae in the Indo-Pacific region (1-7). In *L. nebulosus* in New Caledonia, South Pacific, Rascalou & Justine (2007) reported three species of *Calydiscoides*: *C. difficilis* (Yamaguti, 1953) Young, 1969, *C. duplicostatus* (Yamaguti, 1953) Young, 1969, and *C. terpsichore* Rascalou & Justine, 2007; our specimens unfortunately could not be identified at the species level. A photograph of one of the monogeneans is shown in Fig. 1.

Species of *Encotyllabe* Diesing, 1850 are characterized by thin lateral margins which are turned ventrally. The prohaptor includes a pair of rounded or elliptical, muscular suckers each having a pleated marginal membrane. The opisthaptor has a campanulate disk which is attached to the postero-ventral surface of the body by a long peduncle and is devoid of redial septa, but is equipped with two pairs of hooks and marginal hooklets (8). *Encotyllabe* spp. have already been recorded from the pharyngeal teeth of *Lethrinus* spp. (Justine, 2007), but not identified at the species level. A photograph of this monogenean is shown in Fig. 2.

We found 30 *Calydiscoides* sp. and 5 *Encotyllabe* sp. in gills of *L. nebulosus.* There are not many researches about effects of these parasites on these commercial fish. However a high infectionrate may affect the respiratory system. These monogeneans are reported for the first time from the Iranian coast of the Persian Gulf.

Fig. 1. – *Calydiscoides* sp.

Fig. 2. – *Encotyllabe* sp.

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Establishing a flatworm ageing model

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Ageing is a gradual and inherently complex process, with almost all aspects of physiology and phenotype undergoing steady modifications (1). For studying the different aspects and possible causes of ageing, diverse methodologies and model organisms are used. In recent research, increased attention is drawn to the role of stem cells and tissue homeostasis. Several studies have already demonstrated that ageing is invariably accompanied by a diminished capacity to adequately maintain tissue homeostasis or to repair tissues after injury (1-3). This declining capacity can, however, be caused by several plausible mechanisms, such as age-related changes in the stem cells themselves, in the local environment (niche) in which the stem cells reside, in the systemic milieu of the organism (e.g. the nervous system), or in any combination of these (2). To unravel these distinct contributions to the aged phenotype, more data on these processes is needed. Studying these processes in the existing model organisms is, however, difficult because of the relative inaccessibility of the stem cell population in the vertebrate models on one side, and because of the lack of or limited number of stem cells in adults of models such as *Caenorhabditis elegans* and *Drosophila melanogaster* on the other side (3,4). Therefore, new model organisms should be developed to obtain more data and gain a better insight in this matter.

Free-living flatworms are highly promising organisms in which to unravel such unanswered questions, mostly due to their experimentally-accessible population of likely totipotent stem cells, known as neoblasts (5-7). Furthermore, in several flatworm species, a lifespan extension induced by starvation or repeated regeneration is observed, and several authors even suggest that there is an actual rejuvenation (8-10). This makes it possible to study, not only ageing, but also rejuvenation and the interaction between younger and older tissues. Therefore, flatworms have the potential to become one of the new ageing models we are looking for.

The first step in developing a flatworm ageing model is choosing an appropriate species. An important criterion for making this choice is being able to maintain standardised cultures. This is a necessity for setting up ageing cultures and obtaining enough individuals of a desired age to perform experiments. After trying to culture several flatworm species, we made an initial selection of three species: *Schmidtea mediterranea*, *Schmidtea polychroa* and *Macrostomum lignano*. The next aims were to further standardise the cultures and test the potential of these three species for studying different aspects of ageing. Each species has it strengths and weaknesses, but our experience suggested that *M. lignano* (Macrostomorpha) is the most appropriate for ageing research. Some examples of the advantages of this worm compared to triclads are: 1) a transparent body and well-described anatomy, which makes the in vivo study of cells and organ systems possible during ageing and regeneration, 2) neoblasts and other cell types can be easily labelled by soaking the whole animals in medium containing antibodies, 3) a limited number of cells, 25,000 in total, which makes it possible to quantify neoblasts and other cell types (7,11). Consequently, we chose *M. lignano* to develop it further as a new ageing model. We will therefore focus on this species here.

Culturing *M. lignano* in the laboratory is straightforward and standardised. Individuals are incubated at 20°C and a 13h:11h light: dark cycle (12) and are maintained in f/2, a nutrient-enriched artificial seawater medium at a salinity of 32‰ (13). They are fed ad libitum with the diatom *Nitzschia curvilineata*, which is grown under identical conditions as the worms. Mature worms, which are simultaneous hermaphrodites, generally lay one egg per day and individuals have a short embryonic development of 5 days and a generation time of about 2 – 3 weeks (14).

The next step in initiating ageing research in a new model organism is to obtain a demographic data set, which is essential for designing experiments. These survival data indicate at what age individuals can be considered old and what proportion of the initial cohort is alive at a certain age. This allows one to choose age groups and calculate how big initial ageing cultures should be, in order to give experiments enough statistical power. Besides survival data, knowledge on the age-related changes in mortality rate provides a basic measure for the rate of senescence (15), and can be used to study whether experimental manipulations can alter the rate of ageing or even induce rejuvenation. We have previously followed the survival of *M. lignano* in three replicate cultures consisting of 100 individuals each (16). This experiment has demonstrated that in this species the average (± standard deviation) median lifespan is 205 ± 13 days and

90th percentile lifespan is 373 ± 32 days (16). The maximum lifespan currently observed is 861 days (2.4 years). Further analysis of the survival data allowed us to calculate the mortality rate doubling time (MRDT), which is held to be a fundamental measure of senescence (15,17). *M. lignano* has a MRDT of 0.20 ± 0.02 years and is thus, just as humans, a gradually ageing species according to Finch's (15) classification of senescence patterns. Remarkably, the mammalian models *Mus musculus* and *Rattus norvegicus* have a similar MRDT of 0.27 and 0.30 years respectively (15,17). Despite this, *M. lignano* has a shorter lifespan than these rodent models (maximum lifespan of 4.5 and 5.5 years respectively), which is a clear advantage for experimental work with this species (17). In contrast, other frequently-used invertebrate models such as *C. elegans* and *D. melanogaster* show rapid senescence, with a MRDT of 0.02-0.04 years and a short maximum lifespan of 0.16 and 0.30 years respectively (15). The survival curve itself and a detailed discussion of the above-mentioned demographic data set of *M. lignano* are presented in Mouton et al. (16).

As a next step, we are characterising the morphology as a function of age in *M. lignano*. Characteristic for ageing individuals is the appearance of body deformities, such as a slightly notched epidermis, the presence of grooves in the head region (called urn-shaped invaginations in Ladurner et al. (18)), liquid-filled cysts that tend to be present in all body regions and a disintegration of the gonads in some aged individuals (16,19). These deformities can be an indication of failing cell renewal and tissue homeostasis with advancing age.

To confirm this hypothesis, we conducted preliminary experiments with two concentrations of 5-fluorodeoxyuridine (FUdR): 0.2mM and 0.6mM. FUdR inhibits the cell cycle during DNA replication, which results in the loss of neoblast-functionality and an inhibited tissue homeostasis. Treatment was performed by culturing 1-month old individuals as described above, but with FUdR dissolved in the f/2-medium. Treatment of these young individuals initially resulted in similar deformities as described above, but malformations quickly became more severe and extreme during the progress of the experiment. In the first week of treatment, the epidermis became slightly notched. Later on, the notching of the epidermis became much more extreme. From the second week of treatment, lesions in the epidermis could be observed. Furthermore, organs such as the gonads and the gut started to disintegrate. After the third week, several individuals broke into pieces and died (Fig. 1.). In general, the observed malformations strongly resemble the phenotypes that became apparent following *macpiwi* RNA interference and irradiation of *M. lignano*, which also inhibit neoblast functionality and tissue homeostasis (20,21). An effect of the dosage of FUdR could not be observed by studying the morphology. As this experiment was preliminary, more research is needed to study more FUdR concentrations, recovery after stopping treatment and survival.

Currently we are also studying the number of stem cells and the rate of tissue homeostasis as functions of age. This is possible by labelling S-phase and M-phase neoblasts with the thymidine analog 5'-bromo-2'-deoxyuridine (BrdU) and anti-phosho-histone H3, respectively (7,22).

With the experiments described in this manuscript, we started characterising the phenotypic effects of the ageing process of *M. lignano*. Much more research is needed, but because of several strengths (ease of culturing, accessible stem cell population, available experimental toolbox), this species has the potential to play a crucial role in gaining better insight into the role of stem cell biology and tissue homeostasis for ageing and rejuvenation.

Fig. 1. – Morphology during FUdR-treatment. (A) Morphology of young adults of *M. lignano* in normal culture conditions. (B) Individual during the second week of FUdR-treatment. Several malformations can be observed such as a notched epidermis (bulges indicated with arrows), lesions in the epidermis (*) and a disintegration of the gonads. (C) Individual during the third week of FUdR-treatment. Organs such as the gonads and the gut are completely disintegrated and the epidermis is extremely notched. In all figures, anterior is at the top. A and B are ventral views, C a dorsal view. B: brain, E: eye, G: gut, T: right testis, O: right ovarium, D: developing egg, S: stylet. Scalebars: 100 µm.

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