ISSN- 0102-9010

RODLET CELLS FROM THE GILLS AND KIDNEYS OF TWO BRAZILIAN FRESHWATER FISHES: AN ULTRASTRUCTURAL STUDY

Ivete Mendonça[1], Edilson Matos[2], Gerson Rodrigues[1], Patrícia Matos[3], Graça Casal[4,5,6] and Carlos Azevedo[5,6]

[1]Laboratory of Animal Health (LASAN), Federal University of Piauí, Teresina, PI, Brazil, [2]Carlos Azevedo Research Laboratory, Federal Rural University of Amazonia, Belém, PA, Brazil, [3]Laboratory of Aquatic Animals, Center for Biological Sciences, Federal University of Pará, Belém, PA, Brazil, [4]Department of Sciences, Higher Institute of Health Sciences, 4585-116 Gandra, Portugal, [5]Department of Cell Biology, Institute of Biomedical Sciences (ICBAS), University of Porto, Porto, Portugal, [6]Laboratory of Protoparasitology, Centre for Marine and Environmental Research (CIIMAR), University of Porto, Porto, Portugal.

ABSTRACT

Rodlet cells (RCs) are fish cells considered to be regulatory elements, ion transportation cells, secretory cells, parasitic cells, transport units of genetic material, non-specific immune cells and endogenous in nature cells. In this report, we describe the ultrastructure of RCs collected from the gills and kidneys of two species of freshwater teleosts (family Curimatidae) in Brazil: *Curimata macrops* Eigenmann & Eigenmann, 1889 from the Poty river, near the city of Teresina in the State of Piauí, and *Curimata inornata* Vari, 1989 from the Amazon river near the city of Belém in the State of Pará. A variable number of RCs was observed in these tissues, with a higher frequency in gills compared to the kidneys. No other organs were investigated. RCs were observed in healthy fish and in fish parasitized by a myxosporean of the genus Henneguya. The RCs consisted of a thick-layered capsule enclosing a variable number of small, dense rodlets surrounded by several vacuoles and a nucleus. The capsule was a cytoplasmic structure composed of thick fibrillar elements surrounded externally by the plasmalemma. The capsule and surrounding plasmalemma had a smooth, undulating surface with several microvilli projecting towards the surrounding cells. Some of the microvilli located in the apical zone of the RCs were in contact with the disorganized microfibrils of the capsules. The nucleus was located laterally or basally and showed condensed chromatin at the periphery. The ultrastructural organization of the apical zone of the RCs suggested that these cells may be involved in secretory functions. This is the first report of RCs in these two species of Brazilian fish.

Key words: Freshwater fish, gills, kidney, rodlet cells, ultrastructure

INTRODUCTION

Rodlet cells (RCs) were first described as a parasite by Thélohan [29] and then later redescribed as a protozoan named *Rhabdospora thelohani* [15]. These cells have been reported in freshwater and marine fishes from different geographic regions [4,13,17,19,21,23,26-28]. The nature and functions of RCs have been extensively reviewed elsewhere [21]. RCs have been considered to be parasitic protozoans [3,6,9], pathogenic agents [7,8,25] and normal components of fish tissues [16,23]. These cells are assumed to be the transport units for genetic material [30], and may be involved in immune responses since their number is increased in fish infected with protozoan parasites, particularly at the site of infection. Although RCs have been extensively studied, the precise nature and functions of these cells are still enigmatic and require further study [20,21].

During a routine parasitological analysis [1], some cells identified as RCs were detected in the gills and kidneys of some specimens of two species of Brazilian teleosts, *Curimata macrops* Eigenmann & Eigenmann, 1889 (known locally as "branquinha") and *Curimata inornata* Vari, 1989

Correspondence to: Graça Casal (Doctoral student)
Departamento de Biologia Celular, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Largo Prof. Abel Salazar nº. 2, P-4099-033 Porto, Portugal. Tel. +351.22 206 2200; Fax +351.22 206 2232/33; E-mail: gcasal@icbas.up.pt; azevedoc@icbas.up.pt

(known locally as "coaca pratipioca") (both from the family Curimatidae). In this report, we provide the first description of the ultrastructural organization of these RCs for Brazilian fishes.

MATERIAL AND METHODS

Adult specimens of *C. macrops* and *C. inornata* were collected with a fishing net in the Poty river (5°05´21´´S/42°48´07´´W), near the city of Teresina in the state of Piauí, and in the Amazon river (00º35´38´´S/47º35´00´´W), near the city of Belém in the state of Pará, respectively. The specimens of *C. macrops* were collected in July 2005 and those of *C. inornata* in August of the same year. In the laboratory, the fishes were placed in an aerated glass aquarium (~250 L) for 2-4 h before dissection. The corresponding biometrical data and the number of specimens used are shown in Table 1. The fishes were killed with an overdose of the anesthetic MS 222 (Sandoz Laboratories) diluted in the aquarium water.

For transmission electron microscopy (TEM), small fragments of the gills and kidneys were fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.2, for 10-12 h at 4°C, washed overnight in the same buffer at 4°C, and post-fixed in 2% osmium tetroxide in buffer for 3 h at 4°C. After dehydration in an ascending graded ethanol series and propylene oxide, the tissues were embedded in Epon. Ultrathin sections were cut with a diamond knife, double-stained with uranyl acetate and lead citrate, and examined with a JEOL 100CXII transmission electron microscope operated at 60 kV.

RESULTS

RCs were found more frequently in the gills than in the kidneys of the two fish species. No other organ was investigated. It was not possible to determine the prevalence of the parasite, or to quantatively compare the occurrence of RCs in the two fish species. RCs were distributed randomly among other cell types and were frequently in contact with the surrounding cells. Longitudinal serial sections showed that RCs had an ellipsoidal to oval morphology (Fig. 1) but were circular in cross-section (Fig. 2). Morphometric measurements showed that the RCs were 15.0 ± 1.1 µm (mean ± SD, n = 20) long and 4.2 ± 0.8 µm (n = 25) wide (Fig. 3).

Ultrastructurally, each RC consisted of a plasmalemma in close contact with a thick-layered capsule (Figs. 2-5). The capsules were 0.37 ± 0.45 µm (n = 15) thick and consisted of a dense, compact network of microfibrils that formed a continuous structure in close contact with the internal portion of the cell (Figs. 2, 3 and 5). The periphery of the capsules and the outer membranes of the RCs were irregular, smooth and slightly undulated (Figs. 3 and 5), with microvilli that sometimes projected outwards (Figs. 2 and 3). The apical region of the RCs also showed some microvilli (~0.5 µm long) (Fig. 4). In this region, the microfibrils of the capsules appeared to be disorganized (Fig. 4). No special junctions were observed between the outer membrane of the RCs and the neighboring cells.

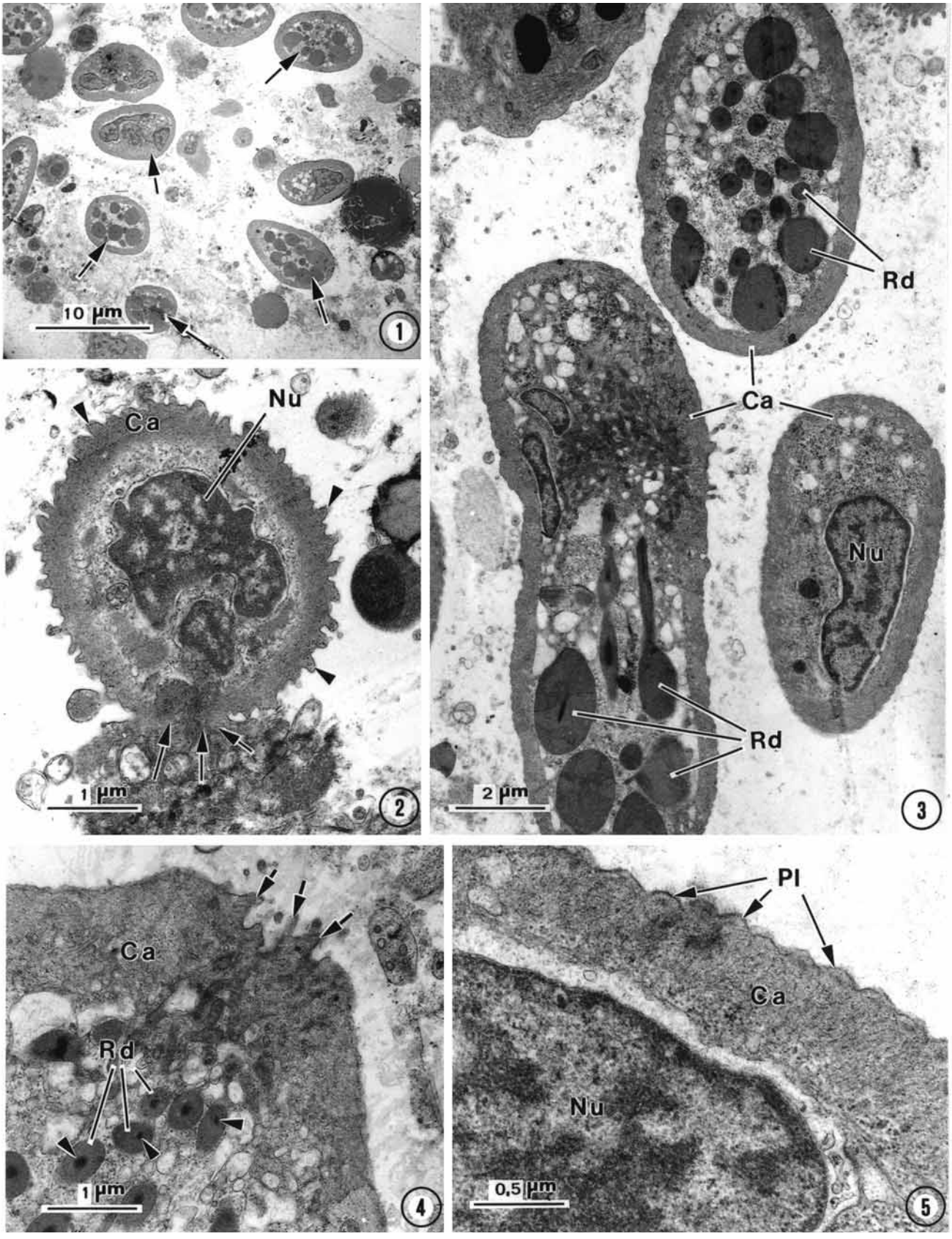
The inner cytoplasm of RCs was occupied by several (up to 15) longitudinal rodlets (club-shaped sacs) surrounded by several light vesicles and vacu-

Table 1. Biometrical and other data for the two Brazilian fish species studied.

Curimata spp.	Total no. of specimens	Total length (cm)	Weight (gr)	Parasitized	Not parasitized	Obs
C. macrops	12	12.5-17	29.5-43	10 (8)**	2 (1)**	(*)
C. inornata	17	9.5-18	21- 41	12 (9)**	5 (4)**	Ref.:[1]

Obs.: (*) *Henneguya* sp.-species not identified yet; ( )** number of specimens containing RCs.

Figures 1-5. Electron micrographs of Rodlet cells. **1.** Dispersed rodlet cells (**arrows**) seen in the gill connective tissue of *C. macrosps* (low magnification). **2.** Transverse section of the basal region of an RC in the kidney of *C. inornata*, showing the periphery of the capsule (**Ca**) with several small microvilli (**arrowheads**), some of which are in close contact with the outer cells (**arrows**). Internally, the nucleus (**Nu**) has an irregular contour and shows condensed chromatin. **3.** RCs in the kidney of *C. inornata* sectioned at different levels, showing the capsule (**Ca**), the rodlets (**Rd**) and the nucleus (**Nu**) of each cell. The periphery of the outer membranes has an irregular appearance (smooth, undulating or the presence of microvilli). **4.** Ultrastructural detail of the apical region of an RC in the kidney of *C. macrops* showing some microvilli projecting out from the capsule (**Ca**) towards the periphery (**arrows**). Internally, some sections of the rodlets (**Rd**) contain dense rods (**arrowheads**). **5.** Ultrastructural detail of the periphery of an RC obtained from the gills of *C. inornata* showing the plasmalemma (**Pl**) and the capsule (**Ca**) with moderate undulations. Part of the nucleus (**Nu**) is also visible.


10 µm
1
Ca
Nu
1 µm
2
Rd
Ca
Nu
Rd
2 µm
3
Ca
Rd
1 µm
4
Pl
Ca
Nu
0,5 µm
5

oles (Figs. 3 and 4). Each rodlet consisted of a peripheral cortex and a central dense core (rod). The cortex formed a club-shaped sac with a fine, granular inner zone and a coarse outer zone (Figs. 3 and 4). In some sections, other cytoplasmic organelles such as the Golgi apparatus, rough endoplasmic reticulum and free ribosomes, were observed (Figs. 3 and 4).

The irregularly shaped nucleus generally occupied a lateral (Fig. 3) or basal (Fig. 2) position in RCs in contact with the capsule (Figs. 3 and 5). The heterochromatin was distributed irregularly in the nucleoplasm (Figs. 2, 3 and 5) and nucleoli were never observed. All RCs were uninucleated cells and were easily observed in serial ultrathin sections.

Most of the RCs were intermingled and in close contact with other surrounding cells of the tissue (Fig. 1). The apical zone of the RCs had several microvilli in close contact with the microfibrils of the capsules that appeared disorganized; in this zone, the internal contents of the RCs were in contact with the apical microvilli (Fig. 4). There were no morphological or ultrastructural differences among the RCs found in the gills and kidneys of the two fish species examined. Figure 6 provides a schematic drawing of the main morphological characteristics of the RCs based on serial ultrathin sections.

DISCUSSION

The RCs described in several fish species from different geographic regions have a similar appearance and the same basic ultrastructural morphology in all of the tissues and species in which these cells have been detected [5-8,14,16,21,25,27,28]. The precise functions of RCs in fish are still unclear, although several controversial functions have been suggested [21]. RCs have been found in a large variety of fish organs and tissues, including the kidney, gill, gut, gall bladder, heart, skin and blood vessel endothelium [8,9,14,21,25]. These cells were initially described as intracellular parasites and named *Rhabdospora thelohani* [29], a conclusion supported by studies in several other fish species [6,24]. In contrast, several studies have suggested that these cells are part of a normal population associated with the defense system of fishes [2,7,8,13,18-21,25]. In agreement with this suggestion, some studies have reported increased numbers of RCs in fish exposed to adverse environmental conditions and toxic substances [12,21].

Various morphological studies have suggested that RCs have a secretory function, at least in some tissues or organs [8,9,11,16,18,19,22,23]. Our results suggested a secretory function for the RCs of the two species examined here based on the ultrastuctural organization of the apical region that showed evident alterations of the organization of the apical periphery, including the appearance of microvilli and microfibrilar disorganization of the capsules just beneath the apical zone where the microvilli differentiated. RCs were seen in healthy fish and in fish parasitized by myxosporeans (*Henneguya* sp.), indicating that there was no association between the presence of RCs and the parasite (see [1] and

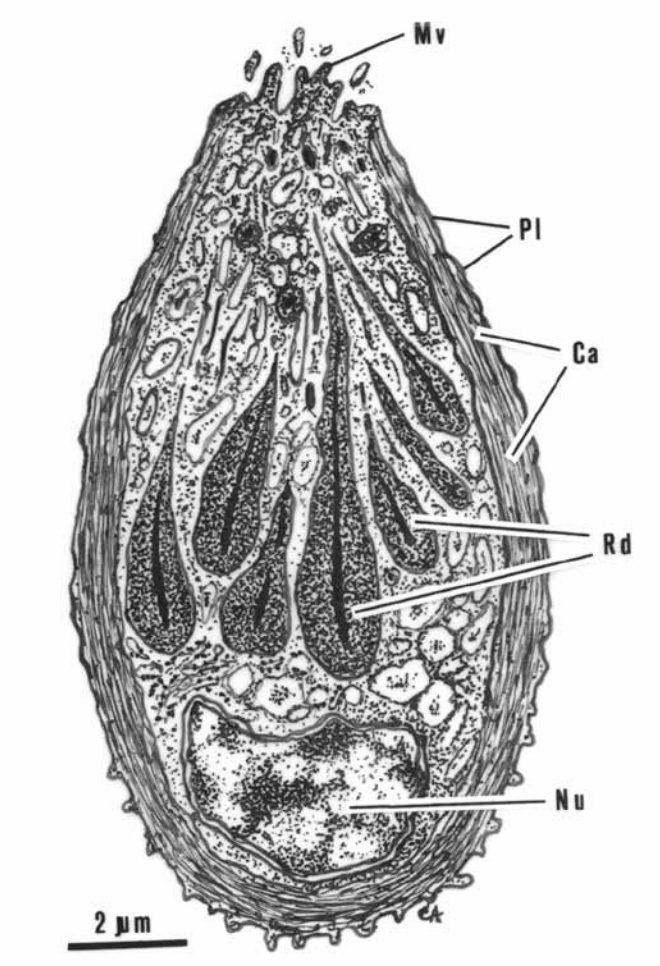


Figure 6. Schematic drawing showing the principal morphological characteristics of a rodlet cell based on serial, longitudinal, ultrathin sections. **Ca** – capsule, **Mv** – apical microvilli, **Nu** – nucleus, **Pl** – plasmalemma, **Rd** – rodlets.

Table 1). Hence, we do not believe that these cells play defensive functions in fish, in contrast to the suggestions of others [17,21,25]. As indicated above, we believe that RCs play a role in secretion.

In conclusion, the ultrastructural analysis described here indicates that the RCs are identical to similar cells from other fishes, and that they are not of parasitic origin, but are normal cells of teleost tissues, and probably have a secretory function, as suggested by others [8,16,20,21]. Nevertheless, various aspects of the origin and function of RCs still remain to be answered [21].

ACKNOWLEDGMENTS

We thank Mr. J. Carvalheiro for the iconographic work and Dr. Jessica Tajdari for revising the English of the manuscript. We also thank the anonymous reviewers for their useful comments and suggestions. This work was partially supported by the Eng°. A. Almeida Foundation (Porto, Portugal).

REFERENCES

1. Azevedo C, Matos E (2002) Fine structure of the myxosporean, *Henneguya curimata* n. sp., parasite of the Amazonian fish, *Curimata inornata* (Teleostei, Curimatidae). *J. Eukaryot. Microbiol.* **49**, 197-200.
2. Balabanova LV, Matey VE (1987) The ultrastructure of rodlet cells from different organs in the carp and brook trout (in Russian with English summary). *Tsitologiya* **29**, 766-770.
3. Bannister HL (1966) Is *Rhabdospora thelohani* (Laguesse) a sporozoan parasite or a tissue cell of lower vertebrates? *Parasitology* **56**, 633-638.
4. Barber DL, Westermann JEM (1975) Rodlet cells in *Catostomus commersoni* (Teleostei: Pisces): secretory cell or parasite? *Experientia* **31**, 924-925.
5. Barber DL, Westerman JEM (1986) Rodlet and histochemistry. *Pathol. Mar. Aquacult.* (*Special Publication*) **9**, 247-258.
6. Barber DL, Westermann JEM, Jensen DN (1979) New observations on the rodlet cell (*Rhabdospora telohani*) in the white sucker *Catostomus commersoni* (Lacépède): LM and EM studies. *J. Fish Biol.* **14**, 277-284.
7. Bielek E (2002) Rodlet cells in teleosts: new ultrastructural observations on the distribution of the cores in trout (*Oncorhynchus mykiss*, *Salmo trutta* L.). *J. Submicrosc. Cytol. Pathol.* **34**, 271-278.
8. Bielek E (2005) Development of the endoplasmic reticulum in the rodlet cell of two teleost species. *Anat. Rec. A Discov. Mol. Cell Evol. Biol.* **283**, 239-249.
9. Bielek E, Viehberger G (1983) New aspects on the rodlet cell in teleosts. *J. Submicrosc. Cytol.* **15**, 681-694.
10. Catton WT (1948) Coarse granulocytes in epithelial tissues of teleost fishes. *Nature (Lond.)* **162**, 894.
11. Desser SS, Lester R (1975) An ultrastructural study of the enigmatic "rodlet cells" in the white sucker, *Catostomus commersoni* (Lacépède) (Pisces: Catostomidae). *Can. J. Zool.* **53**, 1483-1494.
12. Hawkins WE (1984) Ultrastructure of rodlet cells: response to cadmium damage in the kidney of the spot *Leiostomus xanthurus* Lacépède. *Gulf Res. Reports* **7**, 365-372.
13. Imagawa T, Hashimoto Y, Kon Y, Sugimura M (1990) Lectin histochemistry as special markers for rodlet cells in carp, *Cyprinus carpio* L. *J. Fish Dis.* **13**, 537-540.
14. Kramer CR, Potter H (2002) Ultrastructural observations on rodlet-cell development in the head kidney of the southern platyfish, *Xiphophorous maculatus* (Teleostei: Poeciliidae). *Can. J. Zool.* **80**, 1422-1436.
15. Laguesse E (1895) Sur le pancréas du Crénilabre et particulièrment sur le pancréas hépatique. *Rev. Biol. Nord* **7**, 343-363.
16. Leino RL (1974) Ultrastructure of immature, developing, and secretory rodlet cells in fish. *Cell Tissue Res.* **155**, 367-381.
17. Leino RL (1979) Aspects of the fine structure, cytochemistry and distribution of teleost rodlet cells. PhD thesis, Union Graduate School, Cincinnati, OH, USA.
18. Leino RL (1980) Aspects of the fine structure, cytochemistry and distribution of teleost rodlet cells. *Dissertation Abstracts International* **41**, 166.
19. Leino RL (1982) Rodlet cells in the gill and intestine of *Catostomus commersoni* and *Perca flavescens*: a comparison of their light and electron microscopic cytochemistry with that of mucous and granular cells. *Can. J. Zool.* **60**, 2768-2782.
20. Leino RL (1996) Reaction of rodlets cells to a myxosporean infection in kidney of the bluegill, *Lepomis macrochirus*. *Can. J. Zool.* **74**, 217-225.
21. Manera M, Dezfuli BS (2004) Rodlet cells in teleosts: a new insight into their nature and functions. *J. Fish Biol.* **65**, 597-619.
22. Mattey DL, Morgan M, Wright DE (1979) Distribution and development of rodlet cells in the gills and pseudobranch of the bass, *Dicentrachus labrax* L. *J. Fish Biol.* **15**, 363-370.
23. Morrison CM, Odense PH (1978) Distribution and morphology of the rodlet cell in fish. *J. Fish. Res. Board Can.* **35**, 101-116.
24. Mourier JP (1970) Structure fine de *Rhabdospora thelohani* Henneguy, protiste parasite de *Gasterosteus aculeatus* L. *Zeitr. Parasitenk.* **34**, 198-206.

25. Reite OB (2005) The rodlet cells of teleostean fish: their potential role in host defence in relation to the mast cells/eosinophilic granule cells. *Fish Shellfish Immunol.* **19**, 253-267.
26. Rocha E, Monteiro RAF, Pereira CA (1994) Presence of rodlet cells in the intrahepatic biliary ducts of the brown trout, *Salmo truta fario* Linnaeus, 1758 (Teleostei, Salmonidae). *Can. J. Zool.* **72**,1683-1687.
27. Smith SA, Caceci T, Marei HE-S, El-Habback HA (1995a) Observations on rodlet cells found in the vascular system and extravascular space of angelfish (*Pterophyllum scalare scalare*). *J. Fish Biol.* **46**, 241-254.
28. Smith SA, Caceci T, Robertson JL (1995b) Occurrence of rodlet cells and associated lesions in the vascular system of freshwater angelfish. *J. Aquat. Anim. Health* **7**, 63-69.
29. Thélohan P (1892) Sur des sporozoaires indeterminés parasites des poissons. *J. Anat. Physiol. (Paris)* **28**, 163-171.
30. Viehberger G, Bielek E (1982) Rodlet-cells: gland cell or protozoon? *Experientia* **38**, 1216-1218.

Received: November 2, 2005
Accepted: December 6, 2005