—研究論文—
*Scientific Paper*

# Comparative morpho-functional study of the intestine of the Antarctic fish *Notothenia coriiceps* and *Trematomus newnesi* (Nototheniidae): Histology and ultrastructure

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# 南極産魚類 *Notothenia coriiceps*, *Trematomus newnesi* (ノトセニア科) 腸の比較形態—機能学的研究: 組織学と微細構造

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**要旨:** 南極地域の日照時間と日射強度の季節変化は，南極海の生物現存量と基礎生産力に影響を与えている．その結果として，魚類の消化管に，形態学的，生理学的変化を伴うような食物摂取上独特な適応が生ずる．本研究の目的は，2種類の南極産ノトセニア科魚類，底生性で活動度の比較的低い *Notothenia coriiceps*，ならびに半漂泳性の魚である *Trematomus newnesi* の腸の形態—機能学的研究を行うことである．両種とも，食肉性魚類の主な特徴である，割合に大きな胃と短い腸を持っている．腸粘膜は吸収上皮細胞，杯細胞，リンパ球，小桿細胞を含む単層円柱上皮から成っている．中性，酸性の粘液物質を生産する杯細胞は，両種とも腸全域に亘って分布する．*N. coriiceps* の幽門垂，中腸前部，そして *T. newnesi* の中腸にある吸収上皮細胞は脂質吸収細胞としての微細構造上の特徴を示す．蛋白質吸収細胞としての特徴を持つ吸収上皮細胞は，両種ともに，腸の後部に認められる．加えて *N. coriiceps* の腸後部には深いひだがあり，そこには毛細血管が集中し，飲作用により大型分子の活潑な輸送が行われているものと思われる．滲透圧調節は，*N. coriiceps* の場合主として中腸の中，後部でなされ，*T. newnesi* では中腸の全域でなされる．腸の機能的形態における，両種のこれらの差異は *N. coriiceps* が広食性であるのに対し，*T. newnesi* の食物の選択幅が狭いという，2種の食性の差異とエネルギー代謝の差異とに依るものと思われる．

***Abstract***: Seasonal variation in the photoperiod and light intensity in the Antarctic region influences the biomass and primary productivity in the Antarctic Ocean. This results in specialized adaptations for obtaining food, associated with morphological and physiological changes in the digestive tract of fish. The aim of the present study is the morpho-functional study of the intestine of two Antarctic Nototheniidae: *Notothenia coriiceps*, a benthic fish that shows relatively low levels of activity, and *Trematomus newnesi*, a semi-pelagic fish. Both show a proportionally big stomach and a short intestine. Those are the main characteristics of carnivorous fish. The intestinal mucosa is lined by a simple columnar

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epithelium with enterocytes, goblet cells, lymphocytes and rodlet cells. Goblet cells produce neutral and acid mucosubstances along the whole of the intestine, in both species. Enterocytes at the pyloric ceca and the proximal portion of the medium intestine in *N. coriiceps*, as well as the medium intestine of *T. newnesi*, show ultrastructural characteristics of lipid absorption cells. Enterocytes with characteristics of protein absorbing cells were observed along the posterior intestine of both species. In addition, *N. coriiceps* shows deep folds at the posterior intestine and a high concentration of blood capillaries, suggesting that in this region there is active transport of macromolecules by pinocytosis. Osmoregulation seems to occur mainly in the medial and distal portions of the medium intestine in *N. coriiceps*, while in *T. newnesi* it occurs along the entire medium intestine. These differences in intestinal functional morphology in both species can be related to their feeding habits and their energetic metabolism, resulting from different utilization of food nutrients, as *N. coriiceps* is a generalist, while *T. newnesi* ingests a narrow spectrum of food items.

## 1. Introduction

The constantly low temperature of the water, and the seasonal availability of food are determinant factors for life of polar ectotherms and their biological characteristics (Clarke, 1983; Johnston and Battram, 1993). These factors cause changes in biomass and primary productivity in the Antarctic ecosystem (Eastman, 1993). To adjust to this seasonality, Antarctic fish have developed feeding habits and structural mechanisms that enable them to increase food absorption and lipid accumulation during the summer. This accumulated energy is used during winter for gonad maturation and egg production (Clarke, 1988; Johnston and Battram, 1993).

The food composition of adult Antarctic fish is variable, some being specialists and others omnivorous (Hureau, 1994), depending mainly on locally available food (Barrera-Oro and Casaux, 1990; Casaux *et al.*, 1990; Kock, 1992; Linkowski *et al.*, 1983). Generally, the groups that are ingested by most of the fish include algae, bivalves, gastropods, amphipods, isopods, mysidaceans, euphausids, polychaetes and fishes (Everson, 1984).

The stomach content of *Notothenia coriiceps* in Admiralty Bay showed a diet basically of invertebrates (Linkowski *et al.*, 1983; Rios and Fanta, 1998), while Casaux *et al.* (1990) and Moreno and Zamorano (1980) noticed that they ingest also algae. Tests in the laboratory show that their preferential food are fish and krill (Fanta and Meyer, 1998). On the other hand, *Trematomus newnesi* ingests pelagic organisms (Linkowski *et al.*, 1983), showing lower diversity in diet (Casaux *et al.*, 1990; Vacchi and La Mesa, 1995), with preference for amphipods and krill (Fanta and Meyer, 1998). Feeding habit studies of Antarctic fish are usually focused on stomach content analyses. Important adaptive mechanisms can be detected if these habits are related to morphological characteristics (Eastman and DeVries, 1982; Ojeda, 1986) such as body shape (Ekau, 1991), the shape and structure of the mouth (Daniels, 1982; DeWitt, 1971; Casaux *et al.*, 1990), the structure of the gill rakers (Rios and Fanta, 1998) and the structure of photo and chemo-sensorial organs used for food detection and selection (Fanta *et al.*, 1999; Grötzner and Fanta, 1998; Meyer and Fanta, 1998).

Different aspects of the morphology of the digestive tract of Antarctic fish have been described: the organs of the gastrointestinal tract (Eastman and DeVries, 1997) and the histology of the stomach in *Notothenia neglecta* (Freiberger, 1996); the histology of the digestive tract in *Trematomus borchgrevinki* (Eastman, 1975); a comparative morphological and morphometric study of 22 Antarctic fish species (Ojeda, 1986); a comparison of the digestive tract of 25 species of nototheniids (Eastman and DeVries, 1997); and the anatomical and histological characterization of the digestive tract of Bovichthyidae, Nototheniidae and Chaennichthyidae (Korovina, 1986; Korovina and Prirodina, 1986; Korovina *et al.*, 1991a, b). Some functional analyses have also been done: the identification of regulatory peptides and serotonine in the gastrointestinal tract of four Antarctic notothenioids (Tagliafierro *et al.*, 1995); and the absorption of lipids and proteins in the intestine of *N. coriiceps* during the summer (Hernandez-Blazquez, 1996).

The gastrointestinal tracts of fish vary in structure, but the most used classification is that of Bertin (1958): 1) anterior intestine, comprising the esophagus and the stomach; 2) medium intestine, consisting of the pyloric ceca, when present, and the intestine proper; and 3) the posterior intestine or rectum, that is the region posterior to the ileum-rectal valve. According to some authors the intestine of teleosteans can be divided histophysiologically into: 1) a proximal segment, with cells specialized in lipids absorption; 2) a medium segment, responsible for the absorption of macromolecules; and 3) a distal segment, that has the function of absorbing water and electrolytes (Noaillac-Depeyra and Gas, 1976, 1979; Stroband and Debets, 1979; Stroband *et al.*, 1979). Therefore specific ultrastructural characteristics will be associated with these segments, mainly in relation to the absorptive cells (Vernier, 1990).

The present research has the aim to analyze histologically and ultrastructurally the cell types that are present in the intestinal mucosa of two Antarctic fishes, the benthic *N. coriiceps* and the semipelagic *T. newnesi*, and relate it to absorption and digestion.

## 2. Materials and methods

*Notothenia coriiceps* and *Trematomus newnesi* were collected with gill nets in the Admiralty Bay (King George Island, South Shetlands) at depths between 40 and 70 m, during the Antarctic summers of 1995, 1996 and 1997 and autumn and spring 1997.

*N. coriiceps* ($n=16$; standard length 14.5-37.9 cm) were kept in 500 liter tanks and *T. newnesi* ($n=14$; standard length between 7.8-15.8 cm) in 100 liter aquaria, at temperature 0°C and salinity 35 ppt, with a constant photoperiod of 20 hours of light and 4 hours of darkness, at the Brazilian Antarctic Station Comandante Ferraz.

Before the collection of the intestine for morphological studies, all fish fasted for 48 hours. After medullar section, the digestive tract was removed and immediately fixed for 8 to 12 hours in Bouin's fluid or in buffered formalin (Culling *et al.*, 1985) for light microscopy. The samples were routinely processed and included in Paraplast Plus®. Sections of 3 to 5 $\mu$m were stained with Haematoxylin-eosin (HE) (Culling *et al.*, 1985) for general morphology; Picro Sirius (Junqueira and Junqueira, 1983) for detection of collagen fibers, and Periodic Acid-Schiff (PAS) and Alcian blue pH 2.5 and 1.0 (Culling *et al.*, 1985) for the identification of, respectively, neutral, acid and sulfated-acid

glycosaminoglycans and/or glycoproteins. Photographs were obtained with an Olympus PM 10AD photomicroscope.

For scanning electron microscopy (SEM) the samples were fixed at 4°C in Karnovski's fixative (Karnovski, 1965) for almost 2 months. Post-fixation was in Goto (Murphy and Roomans, 1984; Inoué, 1985) and dehydration in an alcoholic series. The critical point was obtained with $CO_2$ in a Balzers CDP 030. It was metalled with a 25-nm layer of gold in a Balzers SCD 050 and photographed in a scanning electron microscope Jeol JSM-5300R.

For transmission electron microscopy (TEM) some samples were fixed in Karnovski (Karnovski, 1965) for 2 months. Post-fixation was in osmium tetroxide 2% in sodium cacodylate buffer 0.2 M and pH 7.2, contrasted with an aqueous solution of uranyle 0.5% with saccharose, dehydrated in an alcoholic series and embedded in Araldite. Semi-thin sections were stained with toluidine blue, and ultra-thin sections were contrasted with lead citrate (Reynolds, 1963) and uranyle acetate 2%. Documentation was obtained in a Jeol JEM 1200 EXII® TEM.

## 3. Results

### *3.1. General anatomy and histology of the intestine*

Pyloric ceca are 5 to 7, finger-like, opening at the pyloric stomach. The intestine of *N. coriiceps* and *T. newnesi* forms three loops, distal to the pyloric sphincter. It extends to the anus, showing an ileo-rectal or pre-rectal valve that marks the transition between the medium intestine and the posterior intestine or rectum (Fig. 1A, B). The intestines of both species show similar general anatomic patterns, as in many of the carnivorous teleosts.

A mucosa, a muscularis and a slender submucosa form the intestinal wall. The submucosa becomes thicker along the rectum in *N. coriiceps*.

The mucosa of the pyloric ceca and the medium intestine show primary folds that decrease in height toward the distal portion of the medium intestine and secondary folds in both species. Secondary folds are ramified in *N. coriiceps* and simple in *T. newnesi* (Fig. 1C, D).

The intestinal epithelium consists mainly of enterocytes and goblet cells (Fig. 5A). They also show numerous rodlet cells, lymphocytes, endocrine cells, and some chloride cells, claviform cells and isolated common chemical sense cells. The enterocytes are columnar cells with elongated basal nucleus and an apical brush border. The structure of the enterocytes is not constant along the intestine, showing different histophysiological characteristics as will be described below. Goblet cells are frequent in the pyloric ceca and increase in density toward the distal portion of the intestine and the rectum in both species.

The muscularis shows two layers of smooth muscle: the internal circular and the external longitudinal. Neural elements that form the Auerbach plexus are seen between the two layers.

In both species the medium intestine ends with the ileum-rectal valve. This valve is a projection of the muscularis, the submucosa and mucosa. The aboral fold is thin and ends at the rectum, being like a funnel with the narrow portion toward the posterior intestine.

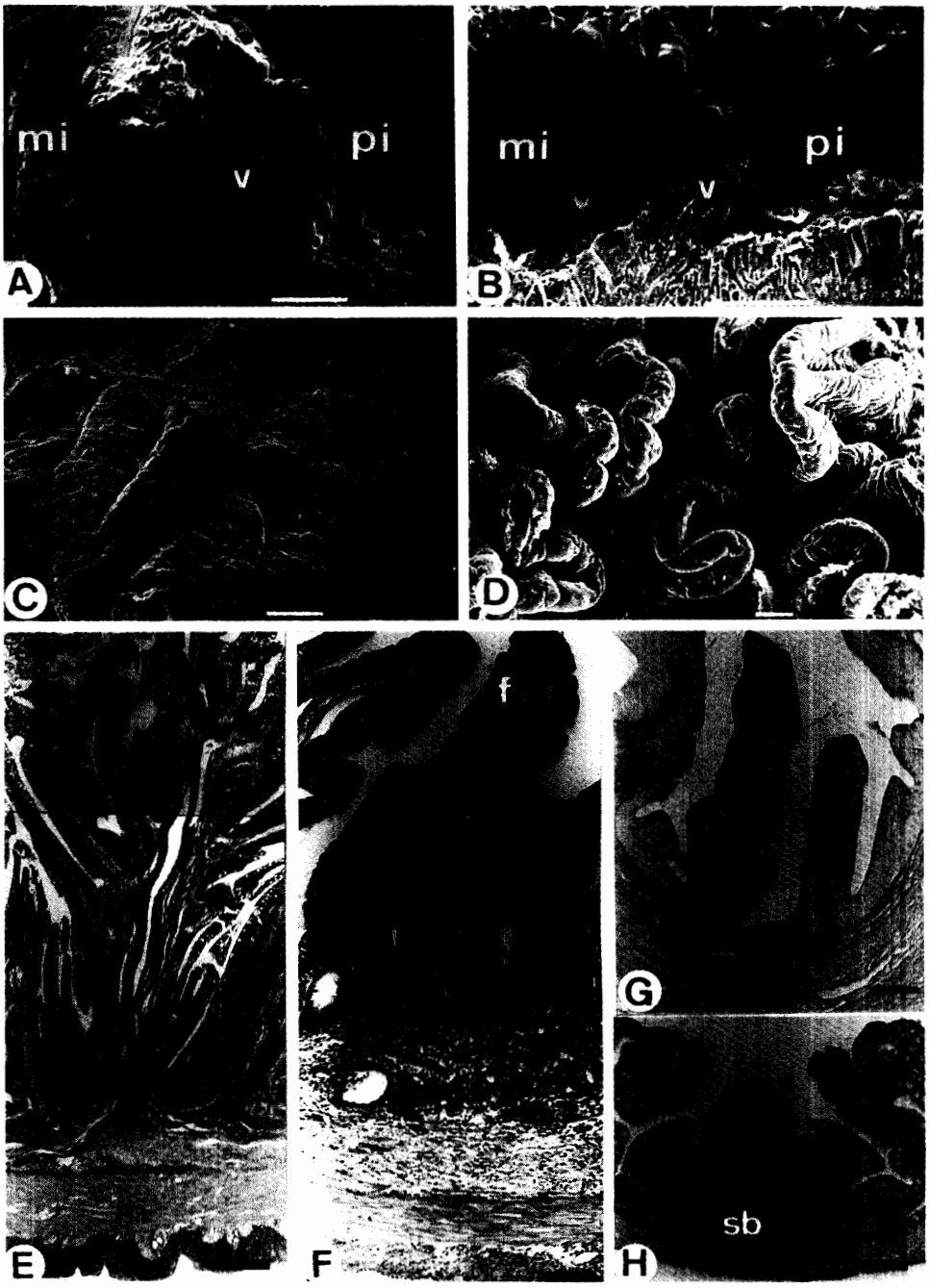


*Fig. 1.* **A**: SEM of the ileum rectal valve (v) between the mid intestine (mi) and the posterior intestine (pi) in *Notothenia coriiceps*, and **B**: the same structure in *Trematomus newnesi*. **C**: SEM of pyloric ceca with broad folds that show secondary folds (f) in *N. coriiceps* and **D**: Twisted leave-like folds (f) on the pyloric ceca in *T. newnesi*. **E**: Median portion of the rectum in *N. coriiceps* with ramified folds (f) touching each other at the intestinal lumen (l). Notice the submucosa (sb) extending into the folds, and the relatively thick muscularis (m). **F**: In *T. newnesi* the same region has fewer folds (f) that are short and mostly sparsely ramified. Notice the thick muscularis of the mucosa (mm). **G**: In the distal region of the rectum of *T. newnesi* the submucosa (sb) and the muscularis (m) are thick and the folds (f) long, but in the proximal region, shown at **H**: the folds (f) are short and the submucosa (sb) and muscular layer (m) are thin. Scales 2 $\mu$m.

The rectum mucosa in *N. coriiceps* is deeply folded and richly vascularized. It has primary and secondary folds that increase their surface. Folds are taller toward the median region, meeting in the lumen (Fig. 1E), but close to the anal sphincter they are shorter and less ramified. *T. newnesi* on the other hand does not show deep and numerous folds in the rectum mucosa (Fig. 1F, G, H).

After the ileum-rectal valve, the muscular and submucosa layers in the rectum of *N. coriiceps* become thicker (Fig. 1E) but in *T. newnesi* this thickening occurs only from the medium region of the rectum onward. Another difference between the two species is that at the distal portion of the rectum of *N. coriiceps* there is still a muscularis of the mucosa.

In both species the rectum ends with the anal sphincter that shows digitiform projections of the mucosa, submucosa and muscularis toward the intestinal lumen. In this region the columnar simple epithelium of the intestine is replaced by a stratified epithelium that is continuous with the skin epidermis.

### *3.2. Histology, histochemistry and ultrastructure of the intestinal cells*

In the intestinal epithelium of both species, eight types of cells were identified histologically, histochemically and through TEM: enterocytes, goblet cells, rodlet cells, lymphocytes, endocrine cells, chloride cells, claviform cells, and isolated common chemical sense cells.

#### **3.2.1.** Enterocytes

The enterocytes presented structural differences along the intestine, both histologically and ultrastructurally, that are related to their function. These differences divide the intestines of both species into segments with different histophysiological characteristics (Table 1).

At the pyloric ceca and the proximal region of the medium intestine in *N. coriiceps*, the enterocytes show electrondense particles, possibly lipid droplets, mainly in their apical region (Fig. 2A). These droplets are observed only in the supranuclear region. Toward the basal region they occur only in the intercellular space and in the adjacent connective tissue (Fig. 2B). These characteristics are also observed in *T. newnesi*, but along the whole medium intestine (Fig. 2C, D, E, F).

The enterocytes of the medial and distal portions of the medium intestine in *N. coriiceps* and of the proximal and medium regions of the medium intestine in *T. newensi* present many baso-lateral invaginations of the plasmatic membrane associated with the concentration of mitochondria (Fig. 3A).

In the rectum of both species the apical cytoplasm of the enterocytes shows structures that are similar to vacuoles, with low affinity to HE (Fig. 3B, C), but with positive reactions to PAS and to Alcian blue pH 2.5 and 1.0.

The enterocytes of the rectum in both species show intense pinocytic activity, due to the presence of many invaginations of their plasmatic membrane at the base of the microvilli (Fig. 3D, E). These invaginations penetrate deeply into the submicrovilli zone and form vesicles of amorphous and weekly electrondense material that can be seen in the apical cytoplasm of the enterocytes of this intestinal segment (Fig. 3D, E). Organelles are scarce in this region of the enterocyte.

In both species, vesicles were less numerous toward the distal portion of the rectum.

*Table 1. Functions of different regions of the intestine in* Notothenia coriiceps *and* Trematomus newnesi. *Comparison with data from literature for tropical (trop.) and temperate (temp.) fish (Ezeasor and Stokoe, 1981; Kirsch and Meister, 1982), and* N. coriiceps *(*N. neglecta*) collected in the Antarctic summer (Hernandez-Blasquez, 1996).*

| | trop. & temp. teleosts | *N. neglecta* Antarctic summer | *N. coriiceps** Antarctic spring | *T. newnesi** Antarctic summer |
|---|---|---|---|---|
| pyloric ceca | abs. lipids | — | abs. lipids | abs. lipids |
| medium intestine | | | | |
| proximal | abs. lipids | abs. lipids | abs. lipids | abs. lipids & osm |
| medial | abs. proteins | abs. lipids | osmoregulation | abs. lipids & osm |
| distal | abs. proteins | abs. lipids | osmoregulation | abs. lipids |
| rectum | | | | |
| proximal | osmoregulation | abs. proteins | abs. proteins | abs. proteins |
| distal | osmoregulation | abs. proteins | abs. proteins | abs. proteins |
| anus | endocrine funct. | | alarm & osm endocrine funct. | chem & osm endocrine funct. |

* shows the results of this paper.
abs. = absorptive function; osm = osmoregulation; alarm = alarm response due to the presence of claviform cells; funct. = function; chem = common chemical sense due to the presence of sensorial cells.

In this region of the intestine the microvilli of the enterocytes are less numerous and shorter (Fig. 3F). In *N. coriiceps* only micropinocytic vesicle invaginations of the plasmatic membrane between microvilli were detected as well as amorphous material in the apical cytoplasm of the enterocytes (Fig. 3F).

The cohesion of the epithelial cells in the distal portion of the rectum in *T. newnesi* is assured by a junctional complex, that is similar to that in the other regions of the intestine, as well as by some isolated desmossomes and interdigitations of the neighboring plasmatic membranes (Fig. 4B). However, in *N. coriiceps* the cohesion of the cells is due only to a juctional complex, great spaces being observed between neighboring epithelial cells, immediately under the junctions (Fig. 3F). In both species the loose connective tissue in the submucosa contains a high concentration of blood vessels (Fig. 4A).

After the anal sphincter the simple columnar epithelium is replaced by a stratified epithelium. Its surface is corrugated and the epithelial cells show microridges that are thick and abundant without definite orientation in *N. coriiceps* (Fig. 4C) but are slender and fingerprint-like in *T. newnesi* (Fig. 4D).

The cells of the apical layer at the anus of *N. coriiceps* and *T. newnesi*, show microridges at their surface and many vacuoles in the cytoplasm. The cytoplasm of these cells is poor in organelles and contains some expanded vesicles, granular reticules and mitochondria, apparently in a stage of degeneration (Fig. 5C) indicating epithelial renewal. Desmossomes and interdigitations of the plasmatic membrane of neighboring apical cells promote their adherence. The basement membrane is thick and the collagen in the underlying connective tissue forms a large net.

**3.2.2.** Goblet cells

There are some histochemical differences in the mucosa of the pyloric ceca, the

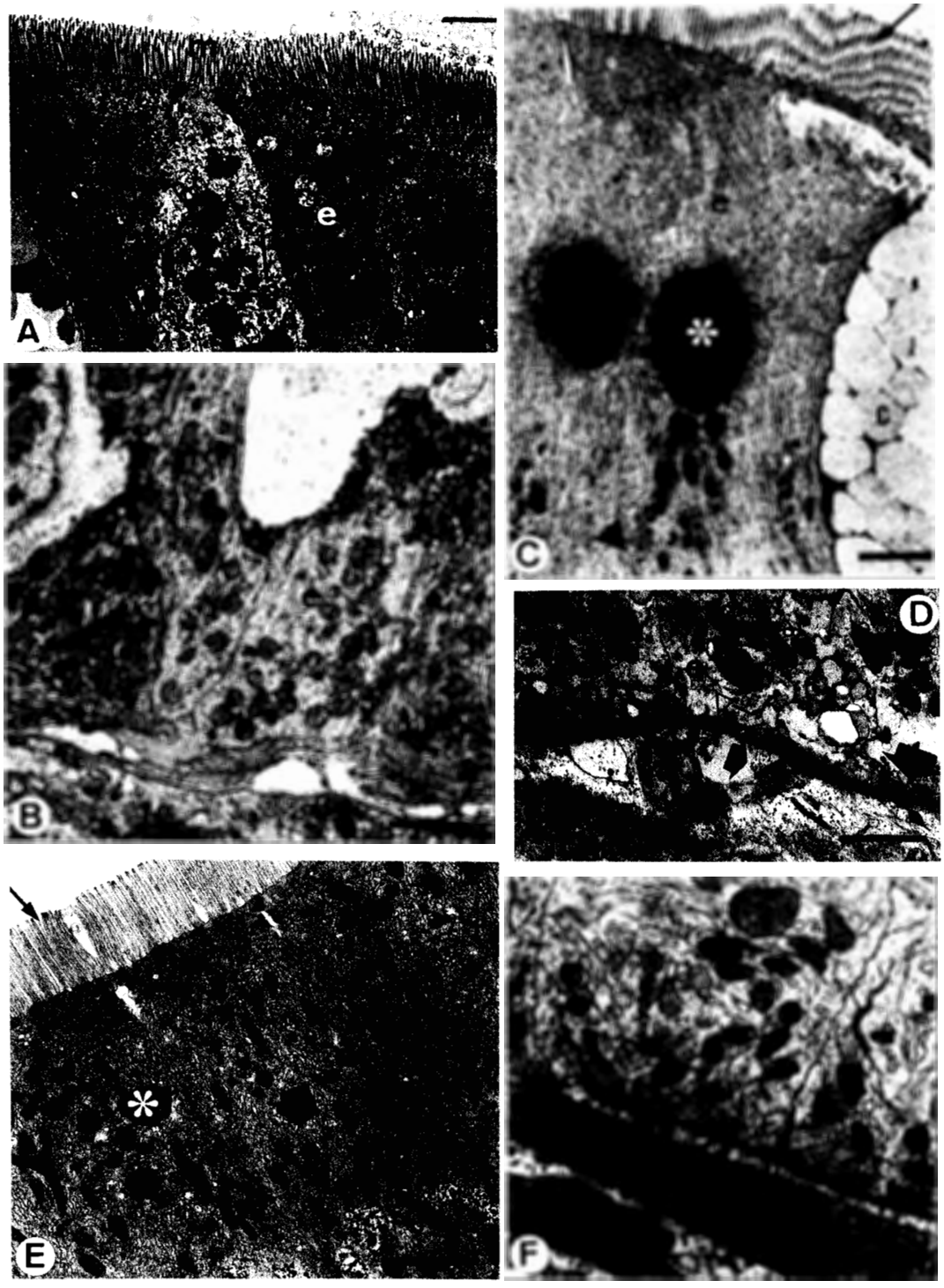


*Fig. 2.*

medium intestine and the rectum between the two species.

In the pyloric ceca, goblet cells produce neutral and acid mucosubstances. But in *N. coriiceps* the positive reaction to PAS was more intense than in *T. newnesi*, where the reaction to Alcian blue pH 2.5 was more intense than in the first mentioned species. Acid mucosubstances rich in sulfated groups were detected only in goblet cells of the pyloric ceca in *T. newnesi* (Table 2).

Goblet cells in the medium intestine and the rectum of *N. coriiceps* produce neutral and acid mucosubstances, but in the proximal portion of the medium intestine they seem to be in different vesicles in the same cell: neutral mucosubstances vesicles are placed in the central portion of the cytoplasm and acid mucosubstances vesicles in the peripheral region. Toward the distal portion of the medium intestine, the goblet cells seem to contain a mixture of both types of vesicles all over the cell. In the medium region of the medium

*Table 2. Histochemistry of goblet cells in different regions of the intestine of* Notothenia coriiceps *and* Trematomus newnesi.

| | *N. coriiceps* | | | | *T. newnesi* | | | |
|---|---|---|---|---|---|---|---|---|
| | Nr | PAS | AB 2.5 | AB 1.0 | Nr | PAS | AB 2.5 | AB 1.0 |
| pyloric ceca | ++ | ++ | + | — | +++ | ++ | +++ | + |
| medium intestine | | | | | | | | |
| proximal | ++ | ++ | ++ | + | +++ | ++ | ++ | + |
| medial | +++ | ++ | ++ | + | +++ | ++ | ++ | + |
| distal | ++++ | ++ | ++ | + | ++++ | ++ | ++ | + |
| posterior intestine | | | | | | | | |
| proximal | ++++ | ++ | ++ | + | ++++ | + | ++ | ++ |
| distal | ++ | +++ | +++ | + | + | + | ++ | ++ |

Nr=proportional amount of cells; PAS= periodic acid-Schiff stain; AB 2.5=Alcian blue pH 2.5; AB 1.0=Alcian blue pH 1.0; number of + indicates the intensity of reaction; — indicates absence of reaction.

*Fig. 2 (opposite).* TEM of medium intestine. **A**: enterocytes (e) of the proximal region in *N. coriiceps*. Notice the presence of lipid droplets (li) and particles (p) in the apical cytoplasm, and microvilli (m), mitochondria (▽), junctional complex (▲) and terminal web (★). **B**: in *N. coriiceps*, detail of the basal region of the epithelium in the proximal medium intestine. Notice lipid particles in the intercellular space (➡) of the enterocytes (e) and free lipid particles (➡) in the subjacent connective tissue (TC) as well as mitochondria (▽), lymphocytes (→) and the basement lamina (▽). **C**: in the distal region of the medium intestine of *T. newnesi* the enterocytes (e) in show microvilli (→), lipid droplets (*) and lipid particles (▲), and mitochondria (▽). Notice a neighboring goblet cell (c). **D**: detail of the epithelium of the proximal medium intestine in *T. newnesi* showing invaginations of the baso-lateral membrane (▽) of the enterocytes (e), lipid particles surrounded by membrane (▲), mitochondria (▽), and free lipid particles in the intercellular space (➡), and also (➡) in the subjacent connective tissue (TC). **E**: enterocytes (e) in the median mid intestine of *T. newnesi*, showing lipid droplets (*) and particles (▲) in the cytoplasm, mitochondria (▽), microvilli (→), and an apical junctional complex (➤). **F**: proximal region of medium intestine in *T. newnesi* showing basement membrane (▽) and beneath it reticular fibers (*). Notice free lipid particles in the intercellular space and entering the basement membrane region (→): Scales: 2 $\mu$m.

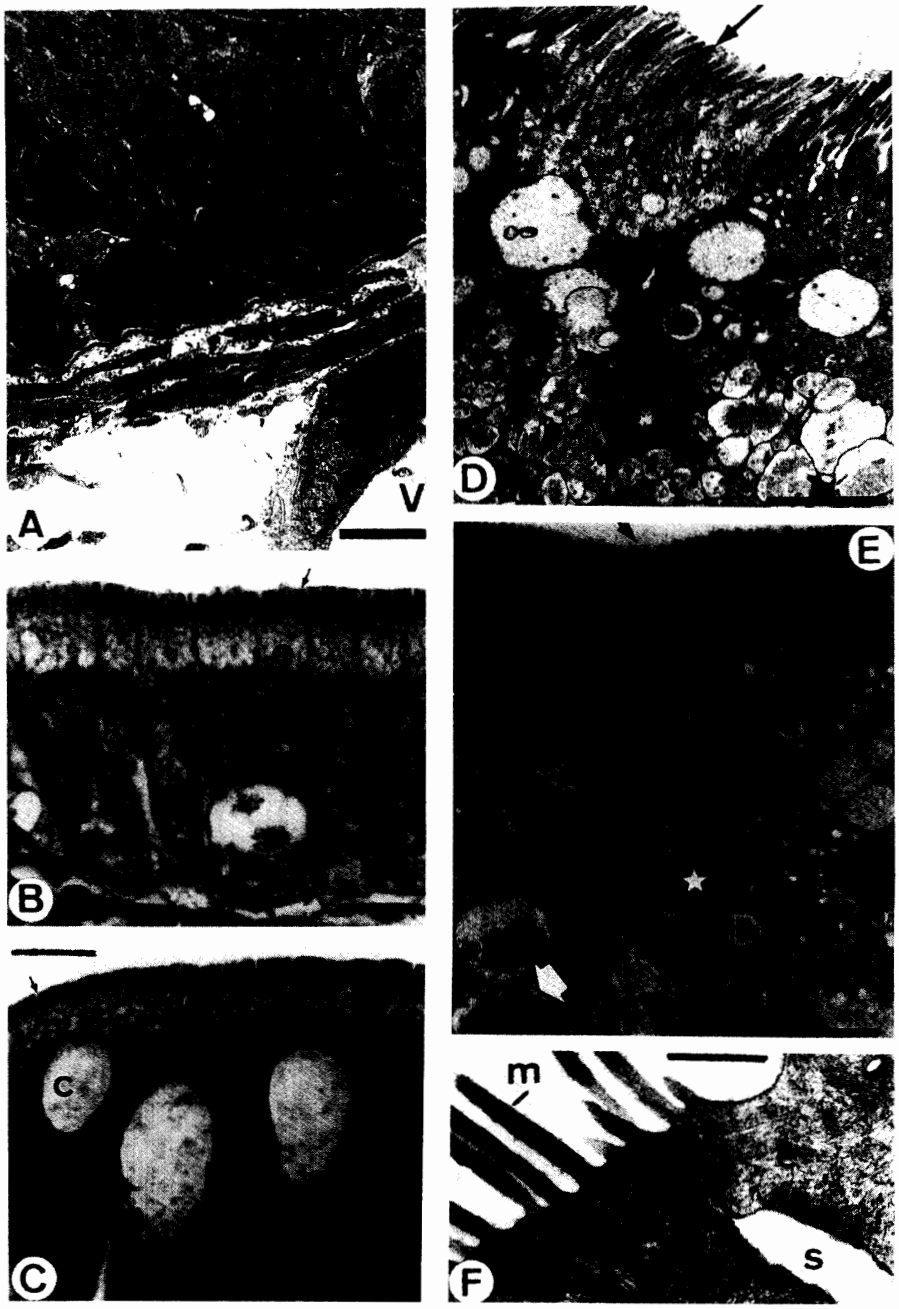


*Fig. 3.* **A**: distal medium intestine in *N. coriiceps* showing baso-lateral invaginations (▽) of enterocytes (e) associated with elongated mitochondria (▽). The connective tissue (TC) contains blood vessels (v). **B**: proximal part of rectum in *N. coriiceps*. Notice the apical cytoplasm of enterocytes (e) with brush border (→), junctional complex (j), and vacuolization (v). The nucleus (n) is basal. **C**: distal part of the rectum in *T. newnesi* with apical vacuolization (v) of enterocytes (e), brush border (→), terminal junctions (j), and a neighboring goblet cell (c). Notice that in **B**, enterocytes show a low affinity for HE, while in **C** there is highly basophylic supranuclear cytoplasm. **D**: rectum in *N. coriiceps* showing enterocytes (e) of the proximal region undertaking pinocytosis (➡) and developing vesicles (v), showing also mitochondria (▽), microvilli (→), and the apical net (☆). **E**: rectum of *T. newnesi* showing enterocytes (e) of the proximal region where pinocytosis (➡) takes place vesicles (v) close to microvilli (→), mitochondria (▽), and also vesicles with amorphous material (☆) or with grannular material (⇨) are seen. **F**: distal portion of the rectum in *N. coriiceps* with detail of enterocytes (e) that present shorter and less numerous microvilli (m). Notice pinocytic vesicles (p), a cytoskeleton rich in filaments (f) and intercellular spaces (s). Scales in **A**, **D** and **E**: 2 $\mu$m **B** and **C**: 10 $\mu$m **F**: 0.5 $\mu$m.

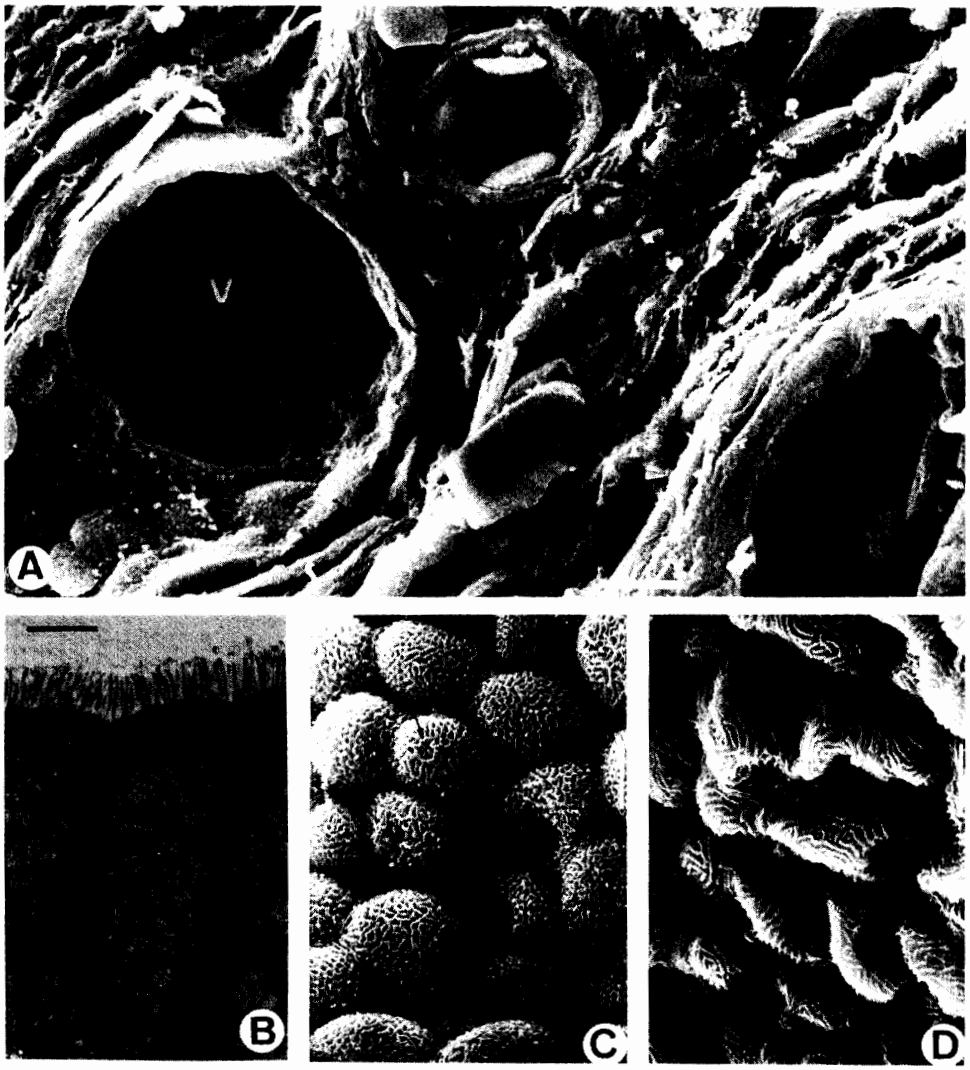


*Fig. 4.* **A**: SEM of the submucosa of the rectum of *T newnesi* showing blood vessels (v) in the loose connective tissue immediately below the epithelium. Scale: 2 $\mu$m. **B**: distal rectum of *T. newnesi* showing the apical cytoplasm of enterocytes (e) where, besides the junctional complex (j), many isolated desmossomes (d) and interdigitations of the membranes of neighbor cells (i) are seen. Notice the high concentration of globous mitochondria (m) and vesicles with amorphous material (v). **C**: SEM of the anal epithelium of *N. coriiceps* showing irregular short microridges (→) at the cell surface. **D**: the same region in *T. newnesi* shows long parallel microridges (→): Scales of **C** and **D**: 5 $\mu$m.

intestine, enterocytes showed a positive reaction to the PAS in the apical region of each cell.

In *T. newnesi*, the goblet cells produce neutral and acid mucosubstances in the same proportion all along the intestine, diminishing in intensity in the rectum. In addition, a positive reaction to PAS and to Alcian blue pH 2.5 was observed in the apical cytoplasm of the enterocytes in the distal portion of the medium intestine. Sulfated acid mucosubstances were detected in the medium intestines of both species. The apical cytoplasm of the enterocytes in the rectum of both, *N. coriiceps* and *T. newnesi* showed positive reaction to PAS and to Alcian blue pH 2.5 and 1.0.

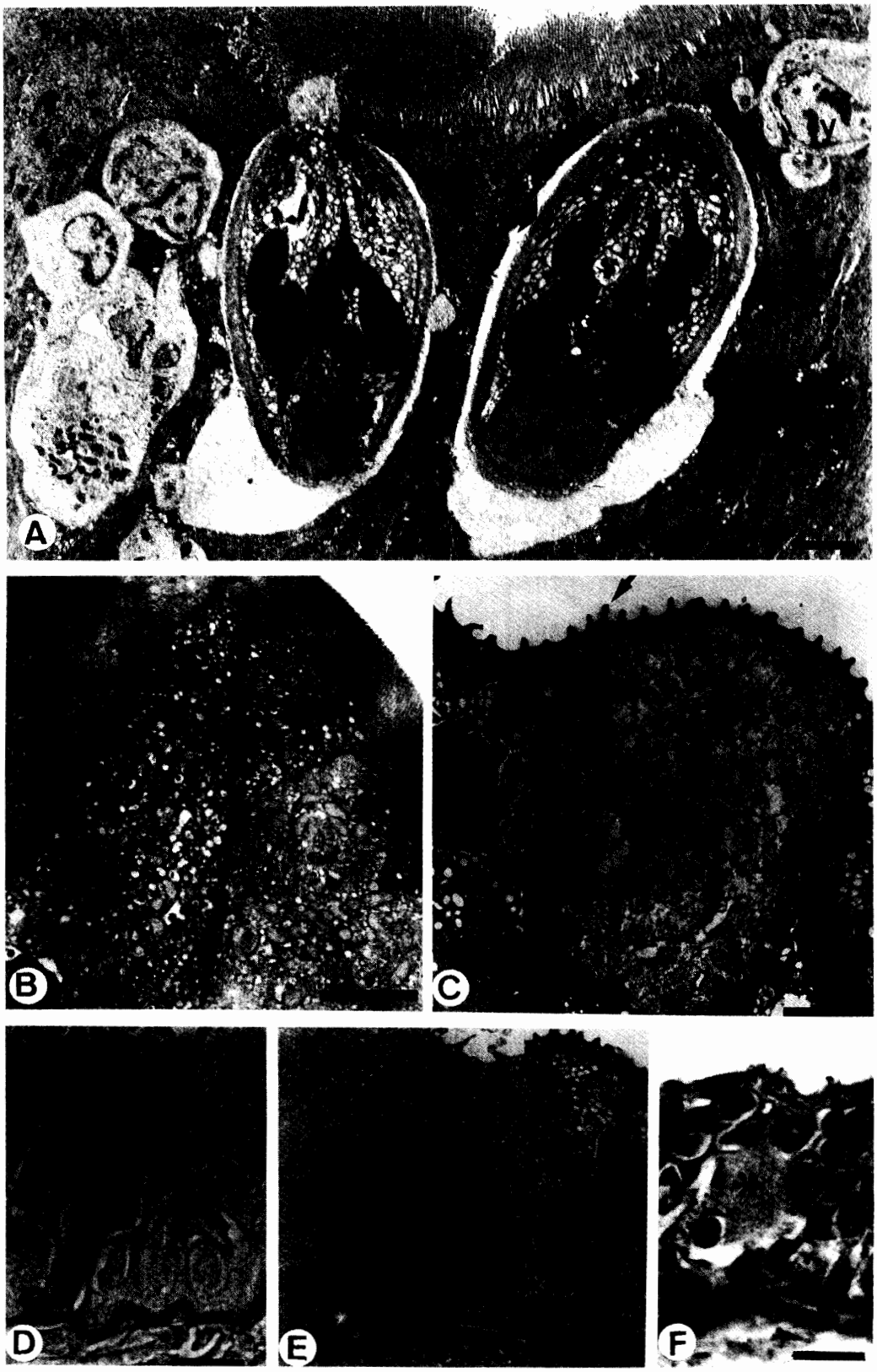


*Fig. 5.*

The superficial cells of the stratified epithelium in the anus of *N. coriiceps* showed a positive reaction to PAS and to Alcian blue pH 2.5, while in *T. newnesi* these cells were positive only to Alcian blue pH 2.5.

**3.2.3.** Rodlet cells

A thick cellular envelope and the presence of cytoplasmic granules and basal nucleus (Fig. 5A) characterize the rodlet cells. The granules, when stained with PAS, present a positive reaction. The cell capsule is formed by the periferic cytoplasm and shows no organelles. Granules are oval with an amorphous external region and a dense fusiform internal region. This cell type was observed in the luminal region as well as in the basal region of the epithelium. The densities of these cells in the epithelium of both species varied with the individuals.

**3.2.4.** Lymphocytes

Lymphocytes are very small cells when compared to the enterocytes (Fig. 5A), with proportionally big dense nucleus. They were seen at various levels of the epithelium. They appear at a low density in the pyloric ceca, the medium and the posterior intestine of both species.

**3.2.5.** Endocrine cells

Endocrine cells lie between the epithelial cells and are characterized by the presence of round secretory granules, some containing a dense central body and others with weakly electron-dense granular material (Fig. 5B).

**3.2.6.** Chloride cells

In the anal epithelium of *N. coriiceps* and between the epithelial cells of the stratified epithelium in the anus of *T. newnesi*, the presence of chloride cells was observed (Fig. 5E). They contain a great number of mitochondria and a vesicle-tubular net in the cytoplasm, resembling the characteristic features of chloride cells in the branchial filaments.

**3.2.7.** Claviform cells

In the anal epithelium of *N. coriiceps*, the presence of claviform cells was observed (Fig. 5F). They are proportionally big roundish cells, with central nucleus and higher cytoplasmic affinity for eosin than the enterocytes.

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*Fig. 5 (opposite).* **A**: TEM of rodlet cells of pyloric ceca in *N. coriiceps*. Notice the typical basal nucleus (N). Notice the absence of organelles in the periferic cytoplasm that forms a capsule like structure (➡): The granules are oval with an amorphous external region (✹) and an internal region that is electron-dense and spindle-like (➢). Notice that one cell is establishing contact with the intestinal lumen (➡). Scale: 2 $\mu$m. **B**: TEM of glandular cells in the medium intestine in *N. coriiceps*. Notice the high concentration of roundish secretory granules with electrondense material (▲) or weak electrondense material (▽). Scale: 15 $\mu$m. **C**: light cells (cl) between dark cells (ce) in the anal epithelium of *T. newnesi*. The light cell shows vacuolized cytoplasm, nucleus (N), desmossomes (→), interdigitation of cell membranes (▽), mitochondria (▽), mitochondria in degeneration (➡), and microvilli (➡). Scale: 2 $\mu$m. **D**: elongated common chemical sense cell (ch), with its basal region in contact with the basement membrane (b) and the apex reaching the epithelial surface (s). Scale: 10 $\mu$m. **E**: pear shaped chloride cell showing a high concentration of supranuclear mitochondria (m) and apical tubule-vesicular net (t) at the anal epithelium of *T. newnesi*. Scale: 2 $\mu$m. **F**. Claviform cell (*) with typical light eosinophyl cytoplasm among the epithelial cells at the anus of *N. coriiceps*. Scale: 10 $\mu$m.

**3.2.8.** Isolated common chemical sense cells

In *T. newnesi* long slender cells that extend from the basement membrane to the surface were observed, showing weak affinity for PAS and high affinity for floxin, resembling the morphological features of the chemo-sensorial cells in the taste buds and of isolated common chemical sense cells seen in the lips of some fish (Fig. 5D).

## 4. Discussion

*N. coriiceps* and *T. newnesi* exhibit the main anatomical characteristics of the digestive tracts of carnivorous teleosts, as has been confirmed for many species of Antarctic fish (Daniels, 1982; Ojeda, 1986): big stomach and short intestine. A short intestine can also be associated with a diet that is rich in nutrients (Dabrowski, 1993). Usually *N. coriiceps* is omnivorous, ingesting various food items, including eventually algae (Barrera-Oro and Casaux, 1990; Casaux *et al.*, 1990; Fanta, 1999; Fanta and Meyer, 1998; Rios and Fanta, 1998; Targett and Radtke, 1984), and *T. newnesi* has a less diversified planktonic diet but is also carnivorous (Casaux *et al.*, 1990; Fanta and Meyer, 1998; Vacchi and La Mesa, 1995). In Admiralty Bay, both species prey mainly on gamariid amphipods but *N. coriiceps* also ingests fish, krill and other invertebrates while *T. newnesi* ingests fewer kinds of organisms (Fanta, 1999; Fanta and Meyer, 1998; Linkowski *et al.*, 1983).

Despite suggestions that there are no differences among the morphology of the digestive tubes in nototheniids (Ojeda, 1986; Matallanas, 1988; Eastman and DeVries, 1997), some differences between the intestine of *N. coriiceps* and *T. newnesi* were found at the ultrastructural, histological and histochemical levels because of slight differences in feeding habits.

If one considers anatomical characteristics, *N. coriiceps* and *T. newnesi* show a relatively constant and low number of pyloric ceca (5 to 7). The relation of these structures and their folds to feeding habits seems to be an increase of the absorptive intestinal epithelium without an increase in intestinal length (Ojeda, 1986), representing an evolutionary advantage. However, in *N. coriiceps* and *T. newnesi*, the presence of numerous and long folds in the pyloric ceca walls, as well as microvilli at the surface of the enterocytes, in addition to the presence of neutral mucosubstances can also be related to an absorptive function, especially of fats, in this portion of the intestine.

There are several differences among *N. coriiceps* and *T. newnesi* in the goblet cells and the mucosubstances that are present along the intestine. In both species a gradual increase in the number of goblet cells was observed from the pyloric ceca toward the rectum. This increase can be necessary to increase the protection of the mucosa as well as to facilitate expelling fecal pellets from the rectum (Grau *et al.*, 1992; Murray *et al.*, 1996).

In *T. newnesi*, and to a lesser extent in *N. coriiceps*, the pyloric ceca have goblet cells that produce acid mucosubstances. Acid mucosubstances, mainly the sulfated ones, are known to inhibit peptic proteases, avoid bacterial infections and protect the mucosa from mechanical actions (Ulibarrie, 1982).

In the proximal region of the medium intestine of *N. coriiceps*, each goblet cell shows separated vesicles for neutral and for acid mucosubstances, while in *T. newnesi* neutral and acid mucosubstances are produced in separate cells that occur in the same proportions

along the whole medium intestine. The coexistence of two or more types of mucosubstances in one cell has been reported for some species as being two or more levels of maturation (Elbal and Agulleiro, 1986; Murray *et al*., 1996). However, this may not apply to the two species studied here, as it has never been observed in *T. newnesi*, while it is consistently observed in *N. coriiceps*. Neutral mucosubstances combined with alkaline phosphatase are responsible for the digestion and emulsification of food (Clarke and Witcomb, 1980), and also provide cofactors required to break enzymes of the food (Anderson, 1986) and help to release energy that is essential for effective absorption and the action of enzymes (Ulibarrie, 1982). Mucous substances are also part of the immunological barrier in the intestinal wall (Pabst, 1987).

Enterocytes show varied specialization in the teleosts intestine, mainly to absorb lipids, proteins and enzymes, and for osmorregulation. The characteristics of lipid absorbing cells are similar in *N. coriiceps* and *T. newnesi*, but not their distribution along the intestine. Lipid absorbing cells were identified because they showed lipid droplets in their cytoplasm and in the intracellular baso-lateral space from where they move, passing through the basal membrane, into the adjacent connective tissue (Murray *et al*., 1996; Noaillac-Depeyre and Gas, 1976, 1979). This transport can be compared to the transport of chylomicron in mammals' mucosa (Cross and Mercer, 1993). Lipid absorbing cells occur in the pyloric ceca of both species, but only in the proximal portion of the medium intestine in *N. coriiceps*, while in *T. newnesi* they occur along the whole of the medium intestine. However, Hernandez-Blazquez (1996) concluded that lipids and proteins are absorbed intensively along the whole medium intestine in *N. coriiceps*. This difference can be because the individuals that he studied were captured during the summer, when feeding activity of this species is the highest, while the individuals studied herein were captured during the spring when the amount of ingested fats is possibly low. Therefore it seems that a wide area was not needed for the absorption of lipids. On the other hand the results found for the medium intestine of *T. newnesi* that were captured during the summer agree with those of Hernandez-Blazquez (1996) for *N. coriiceps*. Therefore one can suggest that the difference in the results for the absorption of lipids in *N. coriiceps* is possibly related to the ingestion of diets that are poor in lipids. Fats play an important role in the energetic metabolism of fish, in the respiratory metabolism, and even in their behavior (Sidell, 1991; Sidell and Crockett, 1987) and some other species, like *Notothenia rossii*, ingest less food during the winter even if enough food is available (Burchett, 1983; Johnston and Battram, 1993). Another hypothesis that can be considered is that the metabolization of lipids in enterocytes of *N. coriiceps*, a benthic and sedentary species, may be slightly lower than for *T. newnesi*, which is a semipelagic and more active species, as a consequence of differences in the energetic metabolism of these species. In the comparison of two ecologically different nototheniids, a higher capacity for the oxidation of fats by the more active *T. newnesi* has been demonstrated when compared to relatively lower capacity in the more inactive *Gobionotothen gibberifrons* (Sidell and Crockett, 1987).

Active transport of water and salts in the intestine of fish is part of hydromineral homeostasis (Jobling, 1995; Loretz, 1995). Enterocytes that may be responsible for osmoregulation varied in their distribution along the intestine for both species. In *N. coriiceps* and in *T. newnesi*, in some enterocytes of the medium intestine the presence of

elongated mitochondria in association with folds of the baso-lateral plasma membrane of the cells indicates energy-dependent activity of these cells, possibly related to osmoregulation (Ezeasor and Stokoe, 1981; Kirsch and Meister, 1982). In *N. coriiceps* these cells occurred only in the medial and distal parts of the medium intestine, while in *T. newnesi* they were found in the proximal and medial parts of the medium intestine. In both species they are also present in the distal part of the rectum, as is also seen in some tropical and temperate fish (Ezeasor and Stokoe, 1981; Kirsch and Meister, 1982).

Protein absorption by enterocytes occurs in the rectum epithelium in both species. In *N. coriiceps* the mucosa of the rectum is deeply folded and highly vascularized, suggesting active transport, and indicating pinocytic entrance and subsequent intracellular digestion of exogenous proteins. This process probably increases the efficiency of protein digestion when emptying of the tract is frequent, due to complete hydrolysis in the stomach (Ezeasor, 1981). In this case the rectal pinocytosis would complement the stomach function. This would be essential in the case of *N. coriiceps*, which feeds on great amounts of food or entire prey, especially in the Antarctic summer. In contrast, the amount of food taken in by *T. newnesi* is smaller, which might be the reason why there are fewer folds in this species and consequently proportionally fewer pinocytic cells. However, supranuclear vacuoles have been observed in apical cytoplasm of the enterocytes in the rectum of *T. newnesi*. These are probably secondary lysosomes or phago-lysosomes, due to the presence of acid hydrolases in their interior, indicating that these cells have the capacity to absorb proteins (Georgopoulou *et al.*, 1985). In addition, the affinity to PAS of the apical cytoplasm of the enterocytes in the distal portion of the medium intestine and in the rectum of both species reflects their lysosomal nature, and can be related to the absorption of macromolecules, as in some other species (Cornell and Padykula, 1969; Georgopoulou *et al.*, 1985; Noaillac-Depeyre and Gas, 1973, 1976, 1979; Sire and Vernier, 1992; Staley *et al.*, 1972; Stroband *et al.*, 1979).

In both species the rectum enterocytes show typical structures for intensive absorption, like long microvilli, and many pinocytic vesicles at their basal region, as well as the presence of great light vesicles and a sub-apical tubule-vesicular net. This coincides with the results found by Hernandez-Blazquez (1996) for *N. coriiceps*. The absorption of proteins by the rectum enterocytes may have the function of reabsorption of anticoagulation proteins that are produced and secreted together with bile and are present in fecal pellets to prevent the formation of ice crystals. Thus, these proteins could be reutilized for economy of energy (Hernandez-Blazquez, 1996). Moreover, the posterior intestine is the only place in the alimentary tract where this absorption can occur without the consequence of dangerous lowering of this anticoagulant. The enzymes produced by the pancreas, stomach and intestine represent another group of macromolecules that could be reabsorbed. The recirculation of pancreatic enzymes through the intestinal epithelium was mentioned by Diamond (1976) for mammals and by Hofer (1982) for fish. This may represent a metabolic economy for both species.

The rectum of both species presented a thickening of the muscularis, which has been related with temporary storage and expelling of fecal material in this region (GRAU *et al.*, 1992). The muscularis of the mucosa separates the lamina propria connective tissue from that of the submucosa in the rectum in *N. coriiceps*. It probably functions as an extra

supporting tissue of the intestinal wall, being an adaptation to the necessity for this species to ingest great and irregular amounts of food. It increases the intestinal motility and facilitates the expelling of fecal pellets. This is not necessary for *T. newnesi*, which take a proportionally smaller amount of food each time they feed.

At the anal sphincter the simple columnar epithelium is replaced by a stratified epithelium whose cells present microridges at their surface, probably to provide a higher level of mechanical protection while holding the mucous secretions at the cell surface (Olson and Fromm, 1973; Schliwa, 1975). They also provide a superficial area for distension (Sperry and Wassersug, 1976). The anal epithelium of both species revealed that the apical cytoplasm of enterocytes presents a large number of vacuoles that can contain acid and neutral glycosaminoglycanes, reactive to Alcian blue pH 2.5 and to PAS, respectively. Therefore one can also suggest a secretory function for these cells with microridges (Olson and Fromm, 1973; Schliwa, 1975).

Rodlet cells are present in the epithelium of numerous organs of freshwater and marine teleosts (Leino, 1974, 1982; Morrison and Odense, 1978; Smith *et al.*, 1995; Freiberger, 1996) and are found both in *N. coriiceps* and *T. newnesi* intestines. They have a morphology that is similar to that of secretory cells, but knowledge about their function and the composition of their granules is still poor. Leino (1982) concluded that the product of these cells is probably neutral, with affinity for PAS, as was here observed. He proposed also that the granules are rich in proteins, but poor in carbohydrates in the central region; and rich in proteins and carbohydrates, probably neutral glycoproteins, at the periphery. These cells seem to multiply in longer periods of starvation, as was observed in the stomach of *N. neglecta* (Freiberger, 1996).

Many intraepithelial lymphocytes are present in the intestinal epithelial layer in *N. coriiceps* and *T. newnesi*, suggesting the existence of a local mucous immune system (Rombout *et al.*, 1989), with regulatory function, suppressing the immune response to intestinal antigens, inducing simultaneously an immune response in the lamina propria (Pabst, 1987).

Endocrine cells were observed along the intestinal epithelium of *N. coriiceps* and *T. newnesi*, but there were no specific differences among them. Elbal *et al.* (1988), Noaillac-Depeyre and Gas (1982), Read and Burnstock (1968), and Rombout (1977) reported endocrine cells in the mucosa of teleostean fish. Tagliaferro *et al.* (1995) detected the presence of many regulatory peptides in the endocrine cells and the nervous elements of the intestine of some species of Antarctic fish. They suggested that these substances control the gastrointestinal absorption, secretion, motility and blood flux.

Chloride cells, similar to those in fish gills (Fanta *et al.*, 1995), were observed in the anal epithelium of *T. newnesi*, suggesting that this region may be actively involved with ionic regulation.

Claviform cells were observed among the epithelial cells of the anus, but only in *N. coriiceps*. The cytoplasmic content of this cell type has not yet been determined chemically; its function seems to be related to alarm substances that mediate intra and interspecific reactions (Downing and Novales, 1971; Henrikson and Matoltsy, 1968; Zaccone *et al.*, 1990). This can be related to the fact that *N. coriiceps* is usually dominant in a fish community where it lives (Fanta, 1999; Fanta and Meyer, 1998).

Isolated cells of common chemical sense were described by Whitear (1952) for teleosts. These cells occur in regions with and without taste buds; they do not belong to the gustatory system, but to a common chemical sense (Whitear, 1971a, b). The same types of cells that are seen in the anal region of *T. newnesi* have been described for this species at the lips (Meyer and Fanta, 1998).

Although the general anatomical and cellular characteristics in Antarctic nototheniids are similar according to Eastman and DeVries (1997), based on the results of the present study one can conclude that there are significant specific differences in the intestinal functional morphology. The differences observed between *N. coriiceps* and *T. newnesi* are closely connected to the ecological characteristics and feeding habits of both.

First, there is the amount and size of food ingested (Fanta and Meyer, 1998; Fanta, 1999; Rios and Fanta, 1998) that explains the development of the muscular layer and the presence of a muscularis of the mucosa in the rectum of *N. coriiceps*, suggesting adaptation to ingestion of great and irregular amounts of food.

One knows also that the nature or the composition of the food is an important factor that affects the evolution of the organs of the digestive system in fish, a systematic group with more than 20,000 species, and that the anterior region of the digestive tract is particularly affected by the diet. On the other hand, the medium intestine of fish with a stomach receives the food significantly digested and basically different in chemical composition and volume (Korovina *et al.*, 1991a).

Thus, it is not a surprise that some significant and noteworthy differences have been observed in the structure and function of the enterocytes, mainly in the medium intestine, between the two nototheniids, *N. neglecta* and *T. newnesi*, but also between them and some tropical and temperate species (Table 2).

If one considers that there are great differences in the necessity of essential nutrients among fish, they must have certain adaptations of the digestive tract and its cells and organelles that are responsible for differential use of the food items. Therefore, the differences that were observed can be related not only to the feeding habit of a certain species but also to its metabolic activity. Thus, the morpho-functional differences that exist between the intestine of *N. coriiceps*, a benthic relatively inactive species, and *T. newnesi*, a more active semi-pelagic species, may be related to differences in their energetic metabolism. Finally, some of the differences can also be the consequence of the season of the year in which individuals were captured, resulting in different use of the essential nutrients that come from the available and ingested food.

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