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Immunocytochemical identification of adenohypophyseal cells in the pirarucu (*Arapaima gigas*), an Amazonian basal teleost

M. I. Borella · R. Venturieri · J. M. Mancera

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Abstract The adenohypophysis (AH) of juvenile pirarucu (*Arapaima gigas*), a representative species of the Osteoglossomorpha (bonytongue fishes, one of the oldest living groups of the teleosts), was studied using histochemical and immunocytochemical methods. The AH is comprised of the pars distalis (PD), without a clear distinction between rostral pars distalis (RPD) and proximal pars distalis (PPD), and the pars intermedia (PI). The neurohypophysis (NH) is positioned on top of the PD and penetrates and branches into the PI. In the most rostral dorsal portion of the PD, adrenocorticotropic cells and fusiform gonadotropic cells were found. In the central PD, scarce prolactin-producing cells and growth-hormone-producing cells were located mainly in the dorsal part, whereas round gonadotropic cells were abundant in the ventral portion of this region. Human thyrotropin immunoreactive cells were not found in the entire AH. In the PI, melanotropic, some adrenocorticotropic, and somatolactin-producing cells were located intermingled surrounding the neurohypophyseal branches. Our results showed that the *A. gigas* pituitary has some basal characteristics between the ancient Actinopterygii and the more derived teleosts.

Keywords Adenohypophysis · *Arapaima gigas* · Bonytongue fish · Immunocytochemistry · Pituitary gland · Ancient teleost · Osteoglossidae

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Introduction

Identification and distribution of adenohypophyseal cells have been studied by immunocytochemistry in several teleosts (Ball and Baker 1969; Holmes and Ball 1974; Agulleiro et al. 2006). Some of these studies have focussed on species of ancient fish groups, including: (1) species of the oldest class of vertebrates, Agnatha (jawless fish), such as lampreys and hagfish (Nozaki et al. 2001, 2005; Kawauchi et al. 2002); (2) representative species of Actinopterygii (subclasses Chondrostei and Neopterygii), such as the *Acipenser* sp. (Grandi and Chicca 2004; Hansen and Hansen 1975; Joss et al. 1990a; Pelissero et al. 1988), *Lepisosteus* sp., and *Amia* sp. (Joss et al. 1990a); and (3) some ancient representatives of the teleost group, such as the eel *Anguilla* sp. and *Synbranchus* sp. (Arakawa et al. 1992; Grandi et al. 2003; Ingleton and Stribley 1977; Vissio et al. 1996). However, to our knowledge, studies that examined the adenohypophysis (AH) of the osteoglossomorphs have not yet been performed.

Osteoglossomorpha is considered an important group because of its place at the base of the phylogeny of teleosts, being a link between the ancient bony fish and the derivate teleosts (Nelson 1994; O'Neill et al. 1998). Studies concerning the brain–pituitary axis in representative species from this group addressed the general structure of the pituitary gland with emphasis on the neurohypophysis (NH) (Tsuneki 1986; Tsuneki and Nosaki 1989) and the molecular forms of gonadotropin-releasing hormone (GnRH) (O'Neill et al. 1998; Okubo and Aida 2001).

The pirarucu *Arapaima gigas* (superorder Osteoglossomorpha, order Osteoglossiformes, family Osteoglossidae, and subfamily Heterotidinae) is an obligatory air-breathing osteoglossid endemic to the Amazonian basin, considered the largest known freshwater scaled fish, reaching 3 m in length and 250 kg in weight (Salvo-Souza and Val 1990). This species is considered an endangered species by the Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES, Appendix II). It has been overfished as a source of food, and there is enormous commercial pressure impacting their natural stocks. At some Amazonian places, *A. gigas* accounts for almost 50% of all commercialized fish (Queiroz and Sardinha 1999). This species is suitable for culture in captivity, but the availability of fingerlings is still the critical point, as there are not enough studies to support commercial production. Therefore, considering that *A. gigas* is a commercial fish and an endangered species important to the Amazonian biodiversity, as well as an interesting biological model, efforts must be made to understand the different aspects of the biology of this species.

In this study, the AH of juvenile specimens of *A. gigas* was studied with histochemical and immunocytochemical techniques to identify the adenohypophysary cell types and their respective distribution. In addition, the results were compared with data obtained in more basal fish species in order to discuss some evolutionary aspects concerning the pituitary gland.

Materials and methods

Animals

Six specimens of juvenile *A. gigas* [Schinz (ex Cuvier) 1822] (Table 1) were used in the study. Specimens were held under natural Amazonian weather conditions (temperature 27°C year-round, rainfall from November to February) in earthen ponds (200–1,000 m^2) at Projeto Arapaima, a commercial fish farm located at Almeirim City, Pará State (Brazilian Amazonian region).

Table 1 Animals used in this study

Number	Gender	Size (cm)	Weight (g)
1	Female	134	19,000
2	Female	35	250
3	Female	31	200
4	Female	30	400
5	Male	117	14,000
6	Male	119	16,000

Morphological study

After capture by an appropriate fishing net, fishes were sacrificed with a lethal dose of tricaine methanesulfonate anesthetic (FINQUEL MS-222, Argent) and decapitated. The pituitary gland was dissected, placed in Bouin fixative for 24 h, and dehydrated and embedded in paraffin. Sagittal and transverse sections (6-μm thick) were obtained from most specimens, whereas sagittal serial sections of the pituitary gland associated with the brain were obtained from only one fish (30 cm; 400 g) to study the hypothalamus–hypophysis connection. For histochemical study, sections were stained with hematoxylin-eosin (HE), periodic acid-Schiff (PAS) (McManus 1948), and Mallory trichrome stains (Pearse 1961).

Immunocytochemical study

For immunocytochemical study, tissue sections were immunostained according to the unlabeled enzyme method of Sternberger (1986). The primary rabbit antisera and working concentration used in this study are shown in Table 2. The antisera against salmon (s) prolactin (PRL), growth hormone (GH), somatolactin (SL), β-follicle-stimulating hormone (β-FSH), β-luteinizing hormone (β-LH), and α,β-luteinizing hormone (α,β-LH) were kindly provided by Dr. H. Kawauchi, Kitasato, Japan (see Kawauchi et al. 1983, 1986; Suzuki et al. 1988a, b; Kaneko et al. 1993). Antirecombinant sea bream (sb) GH, and

Table 2 First antisera used in this study

Antisera raised against	Source	Dilution
Chum salmon PRL	Dr. H. Kawauchi	1:10,000
Human $ACTH_{(1–24)}$	Peninsula Laboratories	1:3,000
Chum salmon GH	Dr. H. Kawauchi	1:10,000
Sea bream GH	Dr. M.M. Valdivia	1:1,000
Human β-TSH	NHPP and NIDDK	1:200
Carp α,β-LH	Dr. E. Burzawa-Gerard	1:1,000
Carp β-LH	Dr. E. Burzawa-Gerard	1:8,000
Chum salmon β-FSH	Dr. H. Kawauchi	1:500
Chum salmon α,β-LH	Dr. H. Kawauchi	1:1,000
Chum salmon β-LH	Dr. H. Kawauchi	1:5,000
Bovine α-MSH	Dr. Wendelaar-Bonga	1:3,000
Chum salmon SL	Dr. H. Kawauchi	1:1,000
Sea bream SL	Dr. M.M. Valdivia	1:1,000

PRL prolactin, *ACTH* $corticotropin_{(1–24)}$, *GH* growth hormone, *β-TSH* β-thyrotropin, *α,β-LH* α,β-luteinizing hormone, *β-LH* β-luteinizing hormone, *β-FSH* β-follicle-stimulating hormone, *α-MSH* monoacetyl α-melanotropin, SL somatolactin

SL were kindly provided by Dr. M. Valdivia, Cádiz, Spain (Martínez-Barberá et al. 1994; Astola et al. 1996). Anti-human (h) $corticotropin_{(1–24)}$ ($ACTH_{(1–24)}$) serum was obtained from Peninsula Laboratories (CA, USA). Anti-bovine monoacetyl α-melanotropin (MSH) was kindly provided by Dr. S.E. Wendelaar-Bonga (van Zoest et al. 1989). Anti-carp (c) α,β-LH, and anti-c β-LH sera were kindly provided by Dr. E. Burzawa-Gerard (Dubourg et al. 1985). The anti-h β-thyrotropin (β-TSH) was kindly provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Hormone and Pituitary Program (NHPP) (CA, USA).

All sections were incubated in the primary antiserum for 18 h at 22°C after a first incubation in 0.3% H_2O_2 in Tris buffer, pH 7.8, for 15 min at 22°C in order to inactivate endogenous peroxidase activity. The secondary antiserum (anti-rabbit immunoglobulin (Ig)G raised in goat, kindly provided by Dr. P. Fernández-Llebrez, Málaga, Spain) was used at a dilution of 1:40 for 45 min at 22°C, followed by rabbit peroxidase-antiperoxidase (PAP) complex (Sigma) at a dilution of 1:100 for 45 min at 22°C. Sections were rinsed three times in Tris buffer after H_2O_2, antisera, and PAP incubation. All antisera and the PAP complex were diluted in Tris buffer, pH 7.8, containing 0.7% nongelling seaweed gelatin lambda carrageenan (Sigma), 0.5% Triton X-100 (Sigma), and 0.02% sodium azide (Merck). The reagent of 0.025% 3.3′-diaminobenzidine tetrahydrochloride (DAB) (Sigma) in Tris buffer, pH 7.8 and 0.007% H_2O_2 (Merck) was used as an electron donor in the dark for 15-min incubation at 22°C. Coplin jars were used for incubation in the first and the second antisera, whereas PAP incubation was carried out in a moist chamber. To enhance immunoreaction, 0.04% DAB plus 0.04% ammonium nickel sulfate hexahydrate (Fluka) were used. To monitor the immunoreactive procedure, contiguous sections went through all of the above steps except incubation in the primary antiserum. Also, normal rabbit serum was used instead of primary antiserum. No positive structures or cells were found in these sections.

Results

Structure of the pituitary gland

Juveniles *A. gigas* specimens used in this study varied greatly in length and weight (Table 1), although all specimens displayed immature gonads colonized only by mitotic and early meiotic-stage germ cells. The pituitary gland of all juvenile *A. gigas* (Figs. 1, 2a) was composed by nervous

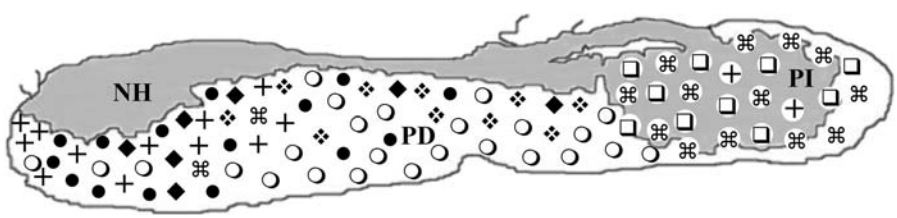

Fig. 1 Representation of sagittal section of the *Arapaima gigas* pituitary showing distribution of adenohypophyseal cell types in juveniles. *PD* pars distalis, *PI* pars intermedia. *Gray area (NH)* neurohypophysis, *white area* adenohypophysis. ◆ Prolactin, + anti-human $corticotropin_{(1–24)}$, ❖ growth hormone, ● putative anti-human β-thyrotropin, ❍ gonadotropin, ⌘ anti-bovine monoacetyl α-melanotropin, ❑ somatolactin-producing cells

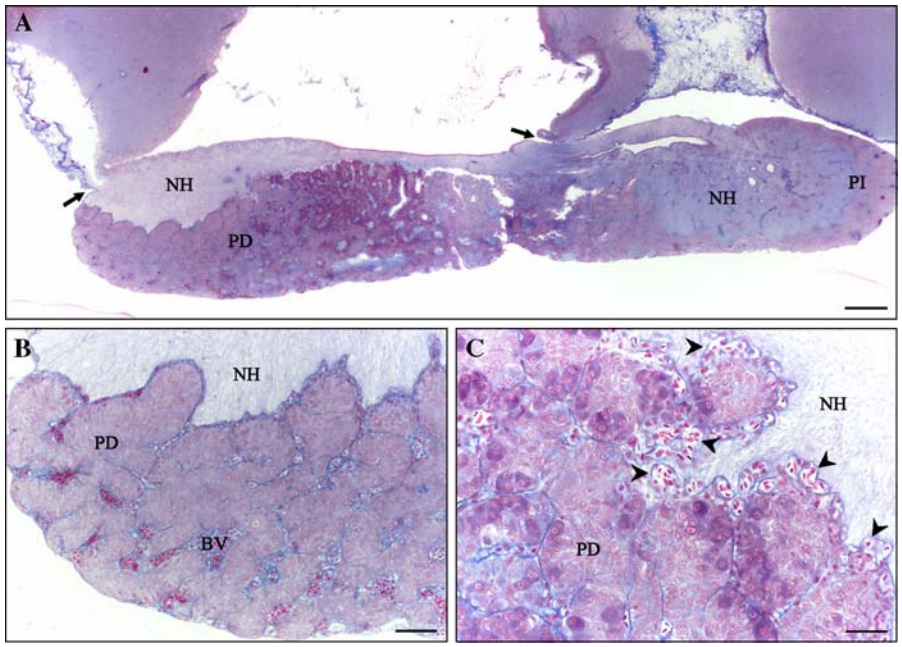

Fig. 2 Pituitary gland of juvenile *Arapaima gigas* stained by Mallory trichrome. **a** Sagittal section showing the pars distalis (*PD*), the pars intermedia (*PI*), the neurohypophysis (*NH*), and the two bounds of nervous tissue that connected the pituitary to the hypothalamus (*arrows*). **b** Anterior part of the PD showing that the NH did not penetrate into the adenohypophyseal tissue. Numerous dilated blood vessels (*BV*) were seen, especially in the ventral part of the adenohypophysis. **c** Detail of the anterior pituitary with the cordonal arrangement of cells and a connective tissue rich in blood capillaries (*arrowheads*) between the PD and the NH. *Scale bars*: **a**, 180 μm; **b**, 48 μm; **c**, 22 μm

tissue, the NH; and glandular tissue, the AH. This last region presented two distinguished parts containing cells organized in clusters and cordonal arrays: the pars distalis (PD) and the pars intermedia (PI) (Figs. 1, 2a). In the PD, cell types were distributed in groups, similar to classical teleost pituitary rostral pars distalis (RPD) and proximal pars distalis (PPD), although the borderline between these parts was not well defined. Many dilated capillaries were seen at the PD among the clusters or cords of cells (Fig. 2b). The NH was disposed upon the PD, and only when reaching the PI did it penetrate and branch into the gland (Figs. 1, 2a). A thin layer of highly vascularized connective tissue could be observed between the NH and the PD (Fig. 2c).

Histochemical techniques (H-E, Mallory trichrome, and PAS) used in this study were useful to understand the pituitary general organization and to distinguish some, but not all, stained cells in the AH. The immunocytochemical techniques revealed in the AH of *A. gigas* at least six different cell types distributed along the entire PD and PI (Fig. 1): (1) ACTH and putative TSH cells in the rostral part of the PD; (2) PRL, GH, and gonadotropic (GtH) cells mostly in the central region of the PD but also in the rostral and caudal PD; and (3) MSH and SL cells in the PI next to the NH branches. In addition, small cell clusters or isolated ACTH cells were found in the PI of *A. gigas* (Fig. 1).

The PRL/GH/SL family

Prolactin cells were not easily recognized when stained with histochemical methods such as Mallory trichrome and were PAS negative. The anti-s PRL used in this study was specific for this cell type (Fig. 3a, b) and did not cross-react with other adenohypophyseal cells. PRL cells were distributed in the central part of the PD close to the rostral area, lining the limit between the NH and the AH, and reaching the ventral PD (Fig. 3c), forming lines or bordering the blood capillaries (Fig. 3a). Scarce PRL cells were also distributed in the caudal PD (Fig. 3c). Most of these cells displayed

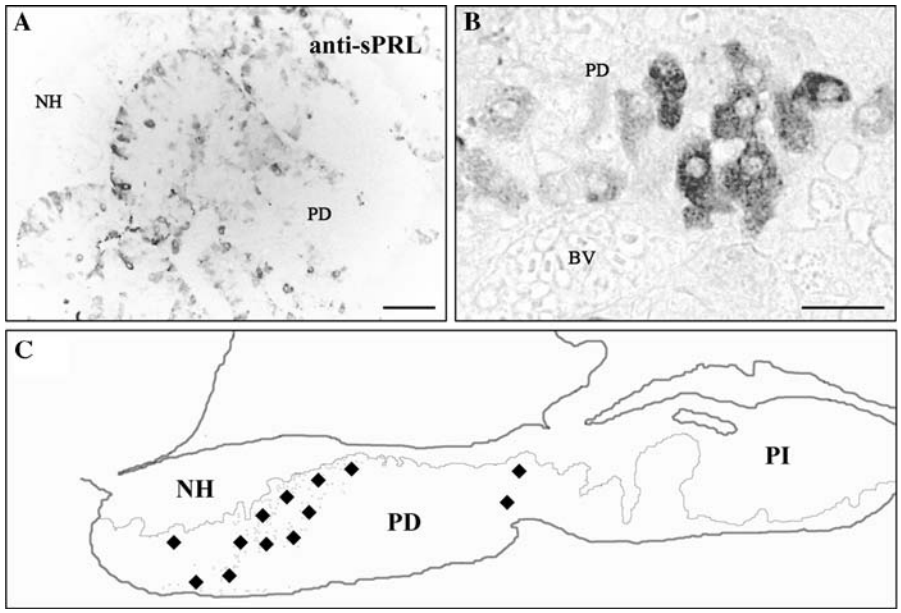

Fig. 3 Sagittal sections of the pituitary gland of juvenile *Arapaima gigas* showing the prolactin (PRL) immunoreactive cells in the PD. **a** PRL cells were found in the central PD, distributed in cords and in thin strands near the NH. **b** Detail of PRL cells close to a BV. **c** Drawing obtained from the original captured image of immunocytochemical reaction using anti-salmon PRL serum. PRL cells (◆) were found in the central PD, mostly lining the rostral PD, from the dorsal to the ventral area. Scarce PRL cells were also distributed in the rostral and caudal PD. *BV* blood vessel, *NH* neurohypophysis, *PD* pars distalis, *PI* pars intermedia. *Scale bars*: **a**, 60 μm; **b**, 23 μm

ovoid shape with a round central nucleus, and some cells were in contact with the blood vessels through cytoplasmic extensions (Fig. 3b).

GH cells were specifically immunostained by anti-s and anti-sb GH antisera employed in this study and did not cross-react with the antisera against PRL or SL. These cells were pale-orange-stained with Mallory trichrome and were PAS-negative. GH-immunoreactive cells were detected along blood capillaries mainly in the dorsal part of the central PD (Fig. 4a, c), but isolated or clustered GH cells were also observed in the ventral part, intermingled with the GtH cells (Fig. 4c). GH cells were elongated with a round or oval-shaped nucleus and frequently displayed cytoplasmic processes that reached blood vessels (Fig. 4b).

Immunoreactive cells to anti-sb SL serum were detected in the PI (Fig. 5a), being negative to antisera developed against other hormones belonging to the GH/PRL family and also to the PAS method. These cells were seen surrounding the neurohypophyseal tissue and blood capillaries (Fig. 5a), most of them at the peripheral areas of the PI. They displayed either polygonal or elongated shape, with round nucleus (Fig. 5b).

The ACTH/MSH family

The anti-h $ACTH_{(1–24)}$ serum strongly immunostained ACTH cells in the PD (Fig. 6a–c) and cross-reacted with putative MSH cells in the PI (Fig. 6c). ACTH cells were arranged in cords in the dorsal part of this region, concentrated mainly just above the anterior neurohypophyseal tissue or in clusters or cords along the capillaries that penetrate the PD (Fig. 6a, b). The ACTH cells were not easily distinguishable from the other cell types by Mallory trichrome stain and were negative to PAS. These cells displayed polymorphic shape occurring as oval, fusiform, or elongated cells, with an ovoid central nucleus. Most labeled cells displayed cytoplasmic processes that reached blood vessels (Fig. 6b).

The anti-b α-MSH serum identified most cells in the PI (Fig. 7a–c), whereas using histochemical methods, MSH cells were PAS negative, and stained weakly blue with the Mallory trichrome method. These cells were distributed forming a net with their cytoplasmic processes in contact with neighboring cells (Fig. 7b), neurohypophyseal tissue, or blood vessels. No adenohypophyseal cells in the PD area

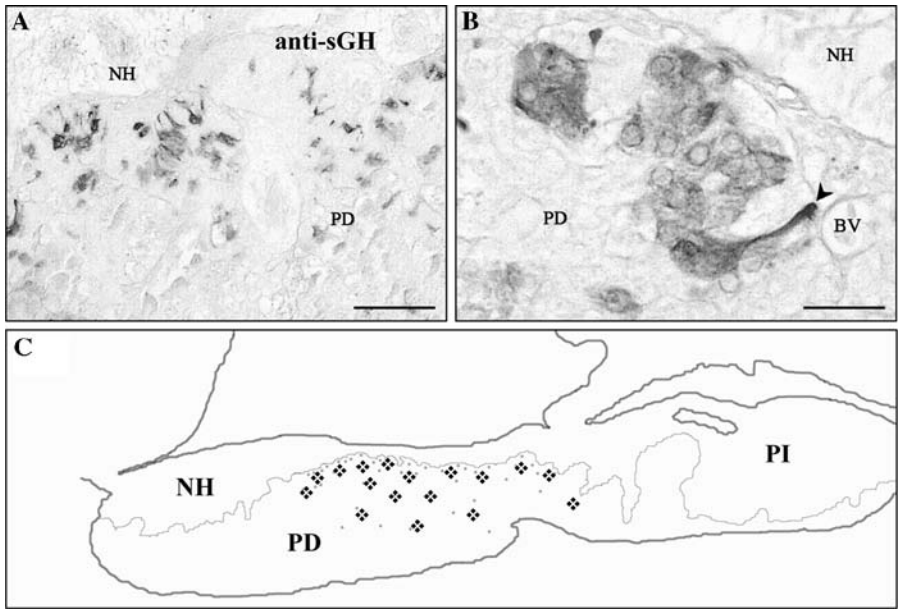

Fig. 4 Sagittal sections of the pituitary gland of juvenile *Arapaima gigas* showing the growth hormone (GH)-immunoreactive cells in the PD. **a** GH cells were seen in the dorsal central PD. Note the NH tissue above the adenohypophyseal tissue. **b** Detail of GH cells forming clusters near the NH. Note the cytoplasmic processes (*arrowhead*) close to a blood vessel. **c** Drawing obtained from the original captured image of immunocytochemical reaction using anti-salmon (s) GH serum in a section consecutive to the immunoreaction to anti-s prolactin (PRL) serum. GH cells (❖) were found in the central PD, mostly in the dorsal area. Scarce GH cells were also distributed in the ventral PD. *BV* blood vessel, *NH* neurohypophysis, *PD* pars distalis, *PI* pars intermedia. *Scale bars*: **a**, 90 μm; **b**, 23 μm

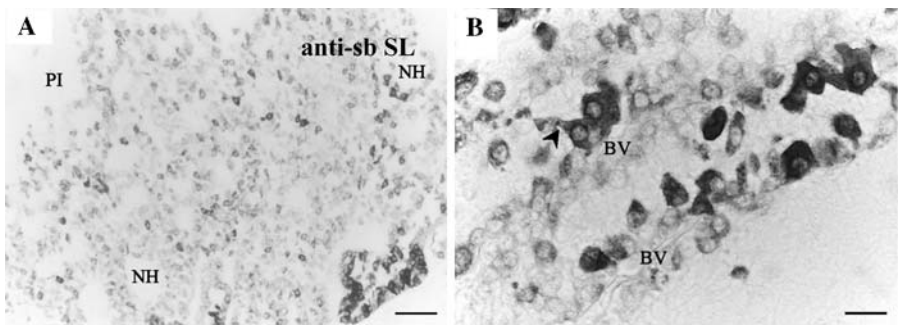

Fig. 5 Sagittal sections of the pituitary gland of juvenile *Arapaima gigas* showing the somatolactin (SL) immunoreactive cells in the PI. **a** Cords and clusters of anti-sea-bream (sb) SL immunoreactive cells in the PI were surrounding neurohypophyseal branches. **b** Detail of SL cells with cytoplasmic processes (*arrowhead*) close to a blood vessel. *BV* blood vessel, *NH* neurohypophysis, *PI* pars intermedia. *Scale bars*: **a**, 48 μm; **b**, 12 μm

correspondent to ACTH cells were immunostained with the anti-α-MSH serum (Fig. 7c).

The GtH/TSH family

Two types of cells, with different shapes, distribution, and immunoreactivity, were observed in the PD when anti-GtH sera were used (Table 3). One type corresponded to a small pyramidal or fusiform cell, strongly immunoreactive to the anti-c α,β-LH serum (Fig. 8a, b) and not easily identified by histochemical methods. These cells were negative to the anti-c β-LH, anti-s β-FSH, anti-s β-LH, and anti-h β-TSH sera used in this study in adjacent sections (Table 2). They were arranged in cords or clusters mainly in the dorsal rostral part of the PD (Fig. 8a, b).

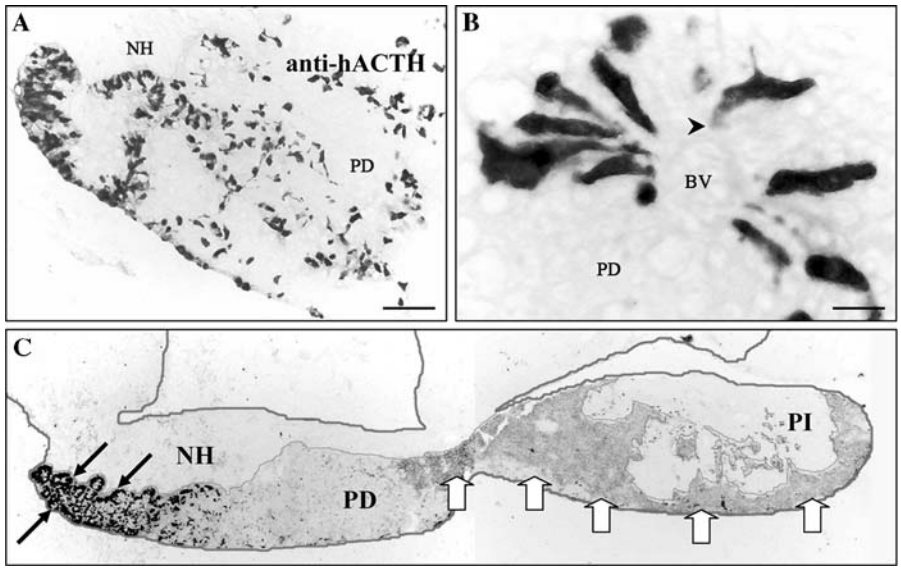

Fig. 6 Sagittal sections of the pituitary gland of juvenile *Arapaima gigas* showing the corticotropin$_{(1–24)}$ (ACTH) immunoreactive cells in the PD. **a** ACTH cells were observed arranged in cords or clusters in the most anterior part of the PD. Note the NH above the adenohypophyseal tissue. **b** ACTH cells with cytoplasmic processes (*arrowhead*) surrounding a blood vessel. **c** Drawing of the original captured image of immunocytochemical reaction using anti-human (h) ACTH serum. Strong ACTH-positive cells (*black arrows*) were found in the rostral PD. Putative MSH cells (*white arrows*) were weakly immunoreactive to the anti-h ACTH serum in the PI. *BV* blood vessel, *NH* neurohypophysis, *PD* pars distalis, *PI* pars intermedia. *Scale bars*: **a**, 60 μm; **b**, 16 μm

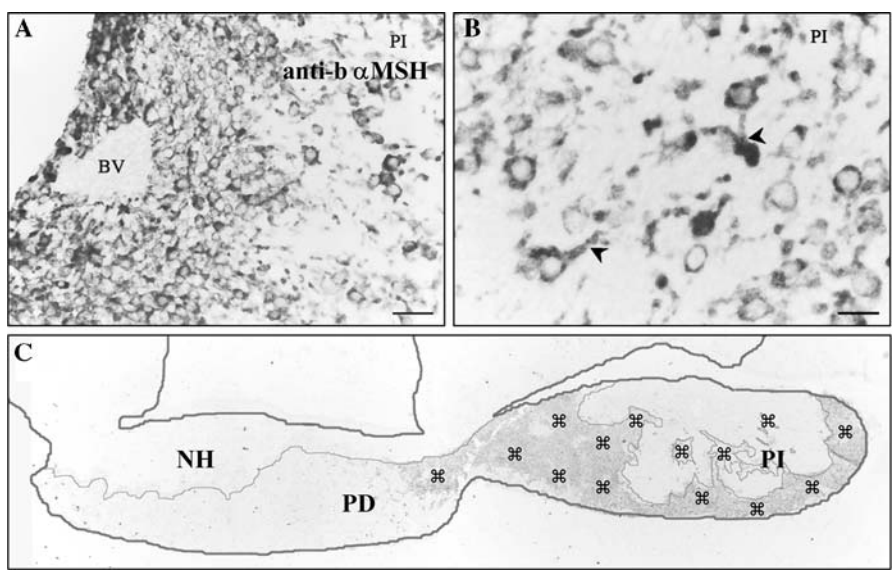

Fig. 7 Sagittal sections of the pituitary gland of juvenile *Arapaima gigas* showing the monoacetyl α-melanotropin (α-MSH) immunoreactive cells in the PI. **a** PI showed numerous anti-α-MSH immunoreactive cells distributed in a net-shaped arrangement, close to a blood vessel. **b** Detail of the cytoplasmic processes (a*rrowheads*) in the PI. **c** Drawing on the original captured image of immunocytochemical reaction using anti-α-MSH serum in a section consecutive to the immunoreaction to anti-h ACTH serum. Note the presence of MSH cells (⌘) in the PI. No cross-reactivity was seen with the corticotropin$_{(1–24)}$ (ACTH) cells in the rostral PD. *BV* blood vessel, *NH* neurohypophysis, *PD* pars distalis, *PI*, pars intermedia. *Scale bars*: **a**, 22 μm; **b**, 12 μm

Table 3 Immunoreaction in adjacent sections of pituitary cells of *Arapaima gigas* to the gonadotropin (GtH) and β-thyrotropin (TSH) antisera used in this study

Antiserum	Fusiform GtH-positive cells	Round GtH-positive cells
Anti-carp α,β-LH	+++	+
Anti-carp β-LH	–	++
Anti-salmon β-LH	–	++
Anti-salmon β-FSH	–	–
Anti-human β-TSH	–	–

α,β-LH α,β-luteinizing hormone, *β-LH* β-luteinizing hormone, *β-FSH* β-follicle-stimulating hormone, *β-TSH* β-thyrotropin

The other cell type (Fig. 8b, c) was larger than the former, displaying round or oval shape, and some of them displaying vacuoles in the cytoplasm. These cells were stained in blue by the Mallory trichrome, being PAS positive. These cells were positive to the anti-c β-LH serum (Fig. 8c) and weakly immunoreactive to the anti-c α,β-LH serum (Fig. 8b) and to the anti-s β-LH serum (data not shown), but they were negative to the anti-s FSH and to the anti-h β-TSH sera used in this study (Table 3). These cells were numerous in the central part of the PD, mostly at the ventral portion. Apparently, they were more numerous and more vacuolated in the larger specimens of *A. gigas* than in the smaller ones, although all the fish used in this study were juveniles. In all used specimens, the anti-h β-TSH and anti-s β-FSH sera did not reveal any cell type in the entire AH.

Discussion

The pirarucu *A. gigas*, endemic to the Amazon region, is one of the largest and more important freshwater fish species alive. This is, to our knowledge, the first study concerning the identification and distribution of the different adenohypophyseal hormone-producing cells in *A. gigas*.

Structure of the pituitary gland

Using histochemical techniques, the NH of osteoglossomorphs, including juveniles of *A. gigas*, showed anatomical characteristics that are intermediate between holosteans such as *Lepisosteus* sp. and nonosteoglossomorph basal teleosts (Tsuneki 1986; Tsuneki and Nosaki 1989). According to these authors, especially in the arowana *Osteoglossum bicirrhosum* and the pirarucu *A. gigas*, which belong to the same family but different subfamilies, a distinct median eminence-like structure was suggested to be present. Our results showed many capillaries between the NH and the PD and dilated blood vessels inside the gland of *A. gigas*. This is not a common feature in most teleost fish AH (Prasada Rao 1969). Moreover, preliminary studies in our laboratory have shown that immunoreactive fibers to the salmon GnRH (sGnRH) were not seen in the pituitary gland of *A. gigas* despite the fact that neuron bodies and fibers were positive in the hypothalamus. Also, blood vessels of the AH appeared immunoreactive to sGnRH. Whether this is suggestive of the occurrence of a blood plexus resembling a hypophyseal-portal system is a matter for further investigation, and further studies with antibodies against other hypothalamic factors should be used to show the presence of a real median eminence in *A. gigas*.

The PRL/GH/SL family

PRL cells were identified mostly in the central PD, but also bordering the rostral part of the *A. gigas* pituitary gland. These cells have previously been demonstrated in the RDP of many other teleosts (Arana et al. 1997; Batten 1986; Parhar et al. 1998; Rodríguez-Gómez et al. 2001; Sánchez Cala et al. 2003; Segura-Noguera et al. 2000), where they were arranged as a compact mass, in follicles, in both, or in thick strands (see Agulleiro et al. 2006). The follicular arrangement of PRL cells present in salmonids and ancient teleost species such as the eel (Arakawa et al. 1992; Grandi et al. 2003; Ingleton and Stribley 1977), which is considered a basal feature, was not found in *A. gigas* pituitary.

As reported for PRL cells of two species of lampreys (Nozaki et al. 2005), PRL cells of *A. gigas* were scarce, arranged in thin strands, and weakly immunostained with the employed antiserum. In addition, these cells were less abundant that one could expect for a freshwater teleost. We can speculate that *A. gigas* PRL molecule shows little affinity to the antiserum raised against the salmon PRL used in this study. Considering the position of the Osteoglossidae at the base of the teleost group, this could be due to a scarce sequence homology of

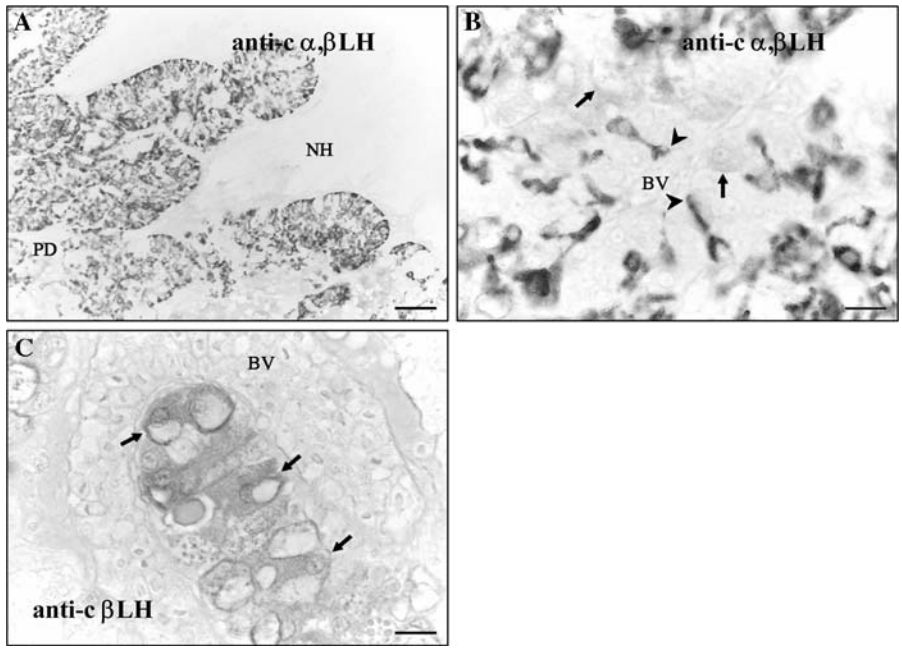

Fig. 8 Sagittal sections of the pituitary gland of juvenile *Arapaima gigas* showing the immunoreactive cells for the **a**, **b** anti-carp α,*β*-luteinizing hormone (anti-c α,*β*-LH) **c** and anti-c *β*-luteinizing hormone (*β*-LH) sera. **a** Dorsal rostral PD showing the strongly anti-c α,*β*-LH-immunoreactive cells distributed in cords bordering the limit of the PD with the NH. **b** These cells displayed a small pyramidal or fusiform shape, with cytoplasmic processes (*arrowheads*) close to blood vessels. In addition, round, weakly anti-c α,*β*-LH immunoreactive cells (*arrows*) were also detected, mostly lining the central PD. **c** In the ventral part of the central PD, large, round, anti-c *β*-LH-immunoreactive cells (*arrows*) with evident vacuoles were close to a blood vessel. These cells and the round cells shown in **b** correspond to periodic acid-Schiff (PAS)-positive cells. *BV* blood vessel, *NH* neurohypophysis, *PD* pars distalis. *Scale bars*: **a**, 48 μm; **b, c**, 12 μm

A. gigas PRL with that of more derivate fish, as well as the sturgeon, another of the basal Osteichthyes, that displays a different molecular form of PRL, with approximately 35–46% sequence similarity with teleost and 30–40% with mammalian PRLs (Noso et al. 1993). Further studies involving cloning of *A. gigas* PRL will be necessary to clarify this subject.

GH cells were identified by both antisera (anti-s and anti-sb GH) used in this study. GH cells were located mainly in the dorsal central PD and were arranged in thin strands in the ventral part of *A. gigas* pituitary gland. This distribution is in agreement with other teleosts, as reported previously (see Ball and Baker 1969; Holmes and Ball 1974; Schreibman et al. 1973) and in more recent studies (Parhar et al. 1998; Rendón et al. 1997; Rodríguez-Gómez et al. 2001; Sánchez Cala et al. 2003; Segura-Noguera et al. 2000; Vissio et al. 1997), although some variations may occur, depending on the species and developmental stage (García-Hernández et al. 1996; Villaplana et al. 2000; Sánchez Cala et al. 2003). These cells were frequently found in teleosts surrounding the neurohypophysial processes of the PPD (see Agulleiro et al. 2006 for references), but this arrangement was not observed in *A. gigas* pituitary, as there are no NH branches penetrating the PD.

GH cells were apparently few in number in *A. gigas* pituitary, and this feature is in agreement with results reported for lampreys (Nozaki et al. 2005). In addition, GH cells showed a weak immunoreactivity with antisera used in the study. These results were not expected, as the animals used in our study were juveniles and were supposed to have a well-developed GH area with very active cells, responsible for growth stimulation. The low cross-reactivity of GH cells might be explained by the phylogenetic distance between *A. gigas* and salmon and sea bream, species from which the used antisera were raised. However, GH-immunoreactive cells were found using a similar anti-chum salmon GH antiserum in at least one species representative of the chondrostean (Grandi and Chicca 2004), a more basal

group than the teleosts. Therefore, more studies involving cloning of *A. gigas* GH are necessary to elucidate this phylogenetic matter.

The SL-immunoreactive cells present in the PI of *A. gigas* pituitary gland were distributed mainly in the peripheral areas of this region, forming cords or clusters surrounding the neurohypophysial tissue and blood capillaries, apparently intermingled with the MSH cells. These results are in agreement with the pioneer findings of this hormone in the cod reported by Rand-Weaver et al. (1991) and for many other teleost species (Kaneko 1996; Laiz-Carión et al. 2003; Sánchez Cala et al. 2003). However, our results showed that, differently from most of teleosts and similarly to salmonids (Agulleiro et al. 2006), the glycosylated form of SL was not found in SL cells of *A. gigas* once they were negative to the PAS method, including in adults (unpublished data).

Similar to our findings in *A. gigas*, cells in the PI of dogfish pituitary were anti-s-SL-serum positive (Kawauchi and Sower 2006), suggesting that SL should display a conservative sequence from more basal to more derived fish. More than one molecular type of SL has been found among teleosts, and also, different molecular forms of SL were cloned from the sturgeon and the lungfish (Amemiya et al. 1999). Whether *A. gigas* has more than one SL molecule is a matter for further investigation.

The ACTH/MSH family

The ACTH amino acid sequence at position 1–24 (of 39 amino acid residues that composes the entire molecule) seems to be constant in mammals (Agulleiro et al. 2006) and in teleosts, as most of the studied species have cells immunoreactive to human $ACTH_{(1–24)}$ antiserum (Borella et al. 1997; Grandi et al. 2003; Laiz-Carión et al. 2003; Rendón et al. 1997; Sánchez Cala et al. 2003; Segura-Noguera et al. 2000). In our study, this antiserum also immunostained ACTH cells at the rostral part of the pituitary. In addition, the antiserum cross-reacted with putative MSH cells located at PI. This could be due to the fact that ACTH and MSH are members of the same proopiomelanocortin (POMC) precursor molecule, where the amino acidic sequence of the α-MSH is identical to the first 13 amino acids of the ACTH molecule (Follénius and Dubois 1980; Dores 1990).

In teleosts, ACTH cells are typically distributed in palisade at the RPD, between PRL cells and branches of neurohypophysial tissue (Ball and Baker 1969; Holmes and Ball 1974; Schreibman et al. 1973). In *A. gigas* pituitary, the NH does not penetrate the PD, and the typical distribution of ACTH cells is not observed. However, and because of their preferential position at the most dorsal position of the rostral part of the PD (next to the NH, but separated from it by a highly vascularized layer of connective tissue), these cells have, in a relative way, a similar pattern of distribution to other teleosts. In addition, few strong putative ACTH-positive cells were seen in the PI intermingled with MSH cells (that showed cross-reactivity with the anti-h $ACTH_{(1–24)}$ serum), suggesting that some isolated ACTH cells were also located in this region. This fact has been previously reported for other teleost species (see Sánchez Cala et al. 2003).

ACTH- and MSH-like cells were found, respectively, in the PD and the PI of two representative species of Agnatha: *Myxine glutinosa* and *Eptatretus burgesi* (Nozaki et al. 2005). Also, in basal actinopterygian representatives (Grandi and Chicca 2004; Joss et al. 1990a, b; Pelissero et al. 1988), these cells were identified in the correspondent pituitary regions. The presence of ACTH, almost without changes in its sequence (Agulleiro et al. 2006), from the most basal vertebrates to the more derived ones, reinforces its physiological importance. In teleosts, besides its classical functions of stimulating cortisol release and stress-response control, ACTH acts in adaptation to hyposmotic environments, and its interactions with other hormones—as with PRL, for example—are under investigation (see Mancera and Fuentes 2006).

The MSH immunoreactive cells were found in the PI of *A. gigas* surrounding the neurohypophysial tissue intermingled with the SL cells, as with the typical distribution of these cells reported for many teleosts (Batten 1986; Cambré et al. 1986; Quesada et al. 1988; Rodríguez-Gómez et al. 2001; Vissio et al. 1997). However, as an arrangement not commonly found among teleosts, these cells were also in clusters, forming a net with their cytoplasmic processes in contact with neighboring cells, and also surrounding blood vessels.

The α-MSH molecule is one of the sequences encoded by the *POMC* gene, together with the ACTH, other MSHs, lipotropins, and β-endorphin

(Mancera and Fuentes 2006). Teleosts MSH cells are generally identified by mammalian α-MSH antisera, once antiteleost MSH serum is not available, and as found in this study, the anti-h ACTH serum also cross-reacts with MSH cells in the PI of other teleosts (Agulleiro et al. 2006).

The presence of the POMC system has been characterized in vertebrates (see Kawauchi and Sower 2006), but in Agnatha such as the hagfish, it is not supposed to be present (Nozaki et al. 2005). However, from basal actinopterygians, including sturgeons (Grandi and Chicca 2004; Joss et al. 1990a; Pelissero et al. 1988), the alligator gar, and the bowfin (Joss et al. 1990a), as well as in the Australian lungfish (Joss et al. 1990b), and from basal teleosts to derived ones (Ball and Baker 1969; Holmes and Ball 1974), MSH cells are present. The results now obtained in *A. gigas* are in accordance with those found for most fish species, suggesting the presence of a POMC system.

The TSH and GtH family

Our results showed two cell types, with different shape and localization, immunoreactive to the applied GtH antisera in *A. gigas* AH. However, none of these cells, or any other cell type, were positive to the anti-s β-FSH or the anti-h β-TSH sera, despite the fact that teleost TSH-β subunits have a high degree of amino acid sequence homology with the human TSH-β subunit (Agulleiro et al. 2006).

Despite the negative response to TSH antiserum, the first GtH cell type described in this study could be a putative TSH-producing cell. The following data supported this idea: (1) they were fusiform, like classical TSH cells of teleosts in general (Ball and Baker 1969; Holmes and Ball 1974); (2) they were distributed in the rostral portion of the PD, as described for TSH cells but not for GtH cells, in some teleosts as *A. japonica* and *A. anguilla* (Ueda et al. 1983; Grandi et al. 2003); and (3) in consecutive serial sections, these cells were positive to the anti-c α,β-LH serum but negative to the anti-c β-LH serum, showing no immunoreactivity to the antiserum raised against the specific β subunit of the GtH. Regarding these last results, antiserum anti-c α,β-LH immunostained both cell types, probably because of the homology between the α chain of the GtH molecule and the presumptive TSH molecule (Kawauchi et al. 1989). However, using the anti-c β-LH, only GtH cells were specifically immunostained because of the reaction of this antiserum with the β subunit of the GtH molecule. Therefore, in consecutive sections, we identified as putative TSH cells those that immunostained with the antiserum anti-c α,β-LH but not with the anti-c β-LH (see Laiz-Carión et al. 2003.

Concerning the presence of TSH cells in basal fish, the literature shows that: (1) immunoreactive cells to anti-h β-TSH and anti-s β-TSH sera were observed in Atlantic and Pacific hagfish *M. glutinosa* and *E. burgeri* (Nozaki et al. 2005); (2) anti-h β-TSH-immunoreactive cells are found in the ventral PPD of larval and juvenile forms of the Italian sturgeon *A. naccarii* (Grandi and Chicca 2004), as are the presumptive TSH cells of the Australian lungfish *Neoceratodus forsteri* (Joss et al. 1990b); (3) in the European eel *A. anguilla*, TSH cells are distributed in the anterior dorsal region of RPD, close to the neurohypophysal branches, around the PRL follicles and among PRL cells, depending on the growth stage (Grandi et al. 2003). Therefore, as it has been observed that the most basal vertebrates and all representatives of basal fishes and teleosts studied present TSH cells—most of which are in a similar position as putative TSH cells found in *A. gigas*—we can strongly assume that these are TSH cells.

The other cell type immunoreactive to GtH antisera used in this study was numerous at the ventral central part of the PD and was larger than the former, with a round or oval shape, some of them with vacuoles in the cytoplasm. These characteristics are in agreement with the typical GtH cells described for several teleosts (Ball and Baker 1969; Holmes and Ball 1974; Van Oordt and Peute 1983; Agulleiro et al. 2006). These GtH cells were positive to the anti-c and anti-s β-LH sera and negative to the anti-s β-FSH serum, suggesting that they could be LH cells. They were also weakly immunoreactive to the anti-c α,β-LH serum. Some features, however, did not allow us to be sure that these cells produce only one hormone homologous to the known LH of teleosts in general, because they showed only weak positive reaction to the LH-employed antisera. Also, the lack of homology between the presumptive FSH of *A. gigas* and the anti-s β-FSH serum could be one explanation of this negative result once FSH appears to be a less conserved molecule (Kawauchi et al.

1989; Swanson et al. 1991). Therefore, we cannot rule out the presence or even the coexistence in the same LH-producing cell type of a second molecular form of GtH in *A. gigas*.

Another reason for not discarding the presence of FSH-like hormone in *A. gigas* is that the analyzed specimens in this work were juveniles, and although the physiological roles of GtH hormones have not been well established for nonsalmonids (Agulleiro et al. 2006) (therefore we do not know exactly when and where the hormones are acting), the number of LH cells exceeded the number of FSH cells in immature specimens of *Thunnus thynnus* (Kagawa et al. 1998) and *Odontesthes bonariensis* (Miranda et al. 2001), and these data could be similar in juvenile *A. gigas*. Further studies using new antibodies against conservative regions of fish gonadotropins should be used in order to understand the pattern of distribution of GtH cells in *A. gigas*.

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References

Agulleiro B, García Hernandéz MP, García Ayala A (2006) Teleost adenohypophysis: morphofunctional and developmental aspects. In: Reinecke M, Zaccone G, Kapoor BG (eds) Fish endocrinology, vol 1. Science Publishers, Enfield, pp 289–323

Amemiya Y, Sogabe Y, Nozaki M, Takahashi A, Kawauchi H (1999) Somatolactin in the white sturgeon and African lungfish and its evolutionary significance. Gen Comp Endocrinol 114:181–190. doi:10.1006/gcen.1998.7250

Arakawa E, Kaneko T, Tsukamoto K, Hirano T (1992) Immunocytochemical detection of prolactin and growth hormone cells in the pituitary during early development of the Japanese eel, *Anguilla japonica*. Zoolog Sci 9:1061–1066

Arana S, Farias EC, Borella MI, Perez ACA, Giamas MTD (1997) Morphological and cytochemical characterization of cells of the adenohypophysis of manjuba, *Anchoviella lepidentostole* (Fowler, 1911) (Osteichthyes, Engraulidae). Braz J Vet Res Anim Sci 34:63–69

Astola A, Pendón C, Ortiz M, Valdivia MM (1996) Cloning and expression of somatolactin, a pituitary hormone related to growth hormone and prolactin from gilthead seabream, *Sparus aurata*. Gen Comp Endocrinol 104:330–336. doi:10.1006/gcen.1996.0178

Ball JN, Baker BI (1969) The pituitary gland: anatomy and histophysiology. In: Hoar WS, Randall DJ (eds) Fish physiology, vol 2. Academic Press, New York, pp 1–205

Batten TFC (1986) Immunocytochemical demonstration of pituitary cell types in the teleost *Poecilia latipinna*, by light and electron microscopy. Gen Comp Endocrinol 63:139–154. doi:10.1016/0016-6480(86)90192-9

Borella MI, Gazola R, Val-Sella MV, Fava-De-Moraes F (1997) Histochemical and immunohistochemical study of the pituitary gland of the South-American teleost pacu fish *Piaractus mesopotamicus* (Cypriniformes, Characidae). Braz J Morphol Sci 14:219–225

Cambré ML, Verdonck W, Ollevier F, Vandesande F, Batten TFC, Kühn ER (1986) Immunocytochemical identification and localization of the different cell types in the pituitary of the seabass (*Dicentrarchus labrax*). Gen Comp Endocrinol 61:368–375. doi:10.1016/0016-6480(86)90222-4

Dores RM (1990) The proopiomelanocortin family. In: Epple A, Scanes CG, Stetson MH (eds) Progress in comparative endocrinology. Wiley-Liss, New York, pp 22–27

Dubourg P, Burzawa-Gerard E, Chambolle P, Kah O (1985) Light and electron microscopic identification of gonadotrophic cells in the pituitary of the goldfish by means of immunocytochemistry. Gen Comp Endocrinol 59:472–481. doi:10.1016/0016-6480(85)90407-1

Follénius E, Dubois MP (1980) Localization of anti-ACTH, anti-MSH, and anti-α-endorphin reactive sites in the fish pituitary. In: Jutisz M, McKerns KW (eds) Synthesis and release of adenohypophyseal hormones. Plenum Publishing, New York, pp 197–208

García-Hernández MP, García-Ayala A, Elbal MT, Agulleiro B (1996) The adenohypophysis of Mediterranean yellowtail, *Seriola dumerilii* (Risso, 1810): an immunocytochemical study. Tissue Cell 28:577–585. doi:10.1016/S0040-8166(96)80060-7

Grandi G, Chicca M (2004) Early development of the pituitary gland in *Acipenser naccarii* (Chondrostei, Acipenseriformes): an immunocytochemical study. Anat Embryol (Berl) 208:311–321. doi:10.1007/s00429-004-0402-5

Grandi G, Colombo G, Chicca M (2003) Immunocytochemical studies on the pituitary gland of *Anguilla anguilla* L., in relation to early growth stages and diet-induced sex differentiation. Gen Comp Endocrinol 131:66–76. doi:10.1016/S0016-6480(02)00646-9

Hansen GN, Hansen BL (1975) Immunohistochemical localization of growth hormone and prolactin in the pituitary gland of *Acipenser güldenstaedti* Brandt (Chondrostei). Acta Zool 56:29–41

Holmes RL, Ball JN (1974) The pituitary gland in teleost fishes. In: Holmes RL, Ball JN (eds) The pituitary gland: a comparative account. Cambridge University Press, London, pp 170–220

Ingleton PM, Stribley MF (1977) Immunofluorescent identification of the cells of origin of Eel (*Anguilla Anguilla*) pituitary hormones separated by polyacrilamyde gel electrophoresis. Gen Comp Endocrinol 31:37–44. doi:10.1016/0016-6480(77)90188-5

Joss JMP, Dores RM, Crim JW, Beshaw M (1990a) Immunocytochemical location of pituitary cells containing

ACTH, α-MSH, and β-endorphin in *Acipenser transmontanus*, *Lepisosteus spatula*, and *Amia calva*. Gen Comp Endocrinol 78:459–468. doi:10.1016/0016-6480(90)90034-J

Joss JMP, Beshaw M, Williamson S, Trimble J, Dores RM (1990b) The adenohypophysis of the Australian lungfish, *Neoceratodus forsteri*—an immunocytological study. Gen Comp Endocrinol 80:274–287. doi:10.1016/0016-6480(90)90172-I

Kagawa H, Kawazoe I, Tanaka H, Okuzawa K (1998) Immunocytochemical identification of two distinct gonadotropic cells (GTH I and GTH II) in the pituitary of bluefin tuna, *Thunnus thynnus*. Gen Comp Endocrinol 110:11–18. doi:10.1006/gcen.1997.7049

Kaneko T (1996) Cell biology of somatolactin. Int Rev Cytol 169:1–24. doi:10.1016/S0074-7696(08)61983-X

Kaneko T, Kakizawa S, Yada T, Hirano T (1993) Gene expression and intracellular localization of somatolactin in the pituitary of rainbow trout. Cell Tissue Res 272:11–16. doi:10.1007/BF00323565

Kawauchi H, Sower SA (2006) The dawn and evolution of hormones in the adenohypophysis. Gen Comp Endocrinol 148:3–14. doi:10.1016/j.ygcen.2005.10.011

Kawauchi H, Abe KI, Takahashi A, Hirano T, Hasegawa S, Naito N et al (1983) Isolation and properties of chum salmon prolactin. Gen Comp Endocrinol 49:446–458. doi:10.1016/0016-6480(83)90208-3

Kawauchi H, Moriyama S, Yasuda A, Yamaguchi K, Shirahata K, Kato J et al (1986) Isolation and characterization of chum salmon growth hormone. Arch Biochem Biophys 244:542–552. doi:10.1016/0003-9861(86)90622-3

Kawauchi H, Suzuki K, Itoh H, Swanson P, Naito N, Nagahama Y et al (1989) The duality of teleost gonadotropins. Fish Physiol Biochem 7:29–38. doi:10.1007/BF00004687

Kawauchi H, Suzuki K, Yamazaki T, Moriyama S, Nozaki M, Yamaguchi K et al (2002) Identification of growth hormone in the sea lamprey, an extant representative of a group of the most ancient vertebrates. Endocrinology 143:4916–4921. doi:10.1210/en.2002-220810

Laiz-Carión R, Segura-Noguera MM, Martín del Río MP, Mancera JM (2003) Ontogeny of adenohypophyseal cells in the pituitary gland of the American shad (*Alosa sapidissima*). Gen Comp Endocrinol 132:454–464. doi:10.1016/S0016-6480(03)00118-7

Mancera JM, Fuentes J (2006) Osmoregulatory action of hypophyseal hormones in teleosts. In: Reinecke M, Zaccone G, Kapoor BG (eds) Fish endocrinology, vol 1. Science Publishers, Enfield, pp 393–417

Martínez-Barberá JP, Pendón C, Rodriguez RB, Pérez-Sánchez J, Valdivia MM (1994) Cloning expression and characterization of a recombinant gilthead seabream, *Sparus aurata*. Gen Comp Endocrinol 96:179–188. doi:10.1006/gcen.1994.1172

McManus JFA (1948) Histological and histochemical uses of periodic acid. Stain Technol 23:99–108

Miranda LA, Strüssmann CA, Somoza GM (2001) Immunocytochemical identification of GtH1 and GtH2 cells during the temperature-sensitive period for sex determination in pejerrey, *Odontesthes bonariensis*. Gen Comp Endocrinol 124:45–52. doi:10.1006/gcen.2001.7687

Nelson JS (1994) Fishes of the world, 3rd edn. Wiley, New York

Noso T, Nicoll RS, Polenov AV, Kawauchi H (1993) Evolutionary implications of the primary structure of sturgeon prolactin. Gen Comp Endocrinol 91:90–95. doi:10.1006/gcen.1993.1108

Nozaki M, Ominato K, Takahashi A, Kawauchi H, Sower SA (2001) Adenohypophyseal cell types in the lamprey: current state of the art. Comp Biochem Physiol B 129:303–309. doi:10.1016/S1096-4959(01)00334-7

Nozaki M, Oshima Y, Miki M, Shimotani T, Kawauchi H, Sower SA (2005) Distribution of immunoreactive adenohypophyseal cell types in the pituitaries of the Atlantic and the Pacific hagfish, *Myxine glutinosa* and *Eptatretus burgeri*. Gen Comp Endocrinol 143:142–150. doi:10.1016/j.ygcen.2005.03.002

O'Neill DF, Powell JFF, Standen EM, Youson JH, Warby CM, Sherwood NM (1998) Gonadotropin-releasing hormone (GnRH) in ancient teleosts, the bonytongue fishes: putative origin of salmon GnRH. Gen Comp Endocrinol 112:415–425. doi:10.1006/gcen.1998.7163

Okubo K, Aida K (2001) Gonadotropin-releasing hormones (GnRHs) in a primitive teleost, the arowana: phylogenetic evidence that three paralogous lineages of GnRH occurred prior to the emergence of teleosts. Gen Comp Endocrinol 124:125–133. doi:10.1006/gcen.2001.7698

Parhar IS, Nagahama Y, Grau EG, Ross RM (1998) Immunocytochemical and ultrastructural identification of pituitary cell types in the protogynous *Thalassoma duperrey* during adult sexual ontogeny. Zoolog Sci 15:263–276. doi:10.2108/zsj.15.263

Pearse AGE (1961) Histochemistry: theoretical and applied. Churchill, London

Pelissero C, Nunez-Rodriguez J, Le Menn F, Kah O (1988) Immunohistochemical investigation of the pituitary of the sturgeon (*Acipenser baeri*, Chondrostei). Fish Physiol Biochem 5:109–119. doi:10.1007/BF01875699

Prasada Rao PD (1969) A comparative study of the pituitary gland of certain freshwater teleosts. Acta Anat (Basel) 73:281–303. doi:10.1159/000143302

Queiroz HL, Sardinha AD (1999) A preservação e o uso sustentado dos pirarucus (*Arapaima gigas*, Osteoglossidae) em Mamirauá. In: Queiroz HL, Crampton WGR (eds) Estratégias para manejo de recursos pesqueiros em Mamirauá. SCM, CNPq/MCT Brasília, pp 108–141

Quesada J, Lozano MT, Ortega A, Agulleiro B (1988) Immunocytochemical and ultrastructural characterization of the cell types in the adenohypophysis of *Sparus aurata* L. (Teleost). Gen Comp Endocrinol 72:209–225. doi:10.1016/0016-6480(88)90204-3

Rand-Weaver M, Baker BJ, Kawauchi H (1991) Cellular localization of somatolactin in the pars intermedia of some teleost fishes. Cell Tissue Res 263:207–215. doi:10.1007/BF00318762

Rendón C, Rodríguez-Gómez FJ, Muñoz-Cueto JA, Piñuela C, Sarasquete C (1997) An immunocytochemical study of pituitary cells of the Senegalese sole, *Solea senegalensis* (Kaup 1858). Histochem J 29:813–822. doi:10.1023/A:1026481521916

Rodríguez-Gómez FJ, Rendón-Unceta MC, Piñuela C, Muñoz-Cueto JA, Jiménez-Tenorio N, Sarasquete C (2001)

Immunocytohistochemical characterization of pituitary cells of the bluefin tuna *Thunnus thynnus* L. Histol Histopathol 16:443–451

Salvo-Souza RH, Val AL (1990) O gigante das águas doces. Cienc Hoje 11:9–12

Sánchez Cala F, Portillo A, Martín del Río MP, Mancera JM (2003) Immunocytochemical characterization of adenohypophyseal cells in the greater weever fish (*Trachinus draco*). Tissue Cell 35:169–178. doi:10.1016/S0040-8166(03)00018-1

Schreibman M, Leatherland JF, McKeown BA (1973) Funcional morphology of teleost pituitary gland. Am Zool 13:719–742

Segura-Noguera MM, Laiz-Carrión R, Martín del Río MP, Mancera JM (2000) An immunocytochemical study of the pituitary gland of the white seabream (*Diplodus sargus*). Histochem J 32:733–742. doi:10.1023/A:1004101127461

Sternberger LA (1986) Immunocytochemistry. Wiley, New York

Suzuki K, Kawauchi H, Nagahama Y (1988a) Isolation and characterization of two distinct gonadotropins from chum salmon pituitary glands. Gen Comp Endocrinol 71:292–301. doi:10.1016/0016-6480(88)90257-2

Suzuki K, Kawauchi H, Nagahama Y (1988b) Isolation and characterization of subunits of two distinct gonadotropins from chum salmon pituitary glands. Gen Comp Endocrinol 71:302–306. doi:10.1016/0016-6480(88)90258-4

Swanson P, Suzuki K, Kawauchi H, Dickhoff WC (1991) Isolation and characterization of two coho salmon gonadotropins, GTH I and GTH II. Biol Reprod 44:29–38. doi:10.1095/biolreprod44.1.29

Tsuneki K (1986) A histologic survey of diencephalic circumventricular organs in teleosts with special reference to osteoglossomorphs. Jpn J Ichthyol 33:27–38

Tsuneki K, Nosaki M (1989) Histological and immunohistochemical studies of the neurohypophysis of primitive teleosts, the Osteoglossidae. Acta Zool 70:47–52

Ueda H, Young G, Nagahama Y (1983) Immunocytochemical identification of thyrotropin (TSH)-producing cells in pituitary glands of several species of teleosts with antiserum to human TSH β subunit. Cell Tissue Res 231:199–204. doi:10.1007/BF00215786

Van Oordt PGWJ, Peute J (1983) The cellular origin of pituitary gonadotropins in teleosts. In: Hoar WS, Randall DJ, Donaldson EM (eds) Fish physiology, vol 9A. Academic Press, New York, pp 137–142

Villaplana M, García Ayala A, Chaves Pozo E, Agulleiro B (2000) Identification of mammosomatotropes, growth hormones cells, and prolactin cells in the pituitary gland of the gilthead sea bream (*Spaurus aurata*. L., Teleostei) using light immunocytochemical methods: an ontogenetic study. Anat Embryol (Berl) 202:421–429. doi:10.1007/s004290000123

Vissio PG, Paz DA, Maggese C (1996) The adenohypophysis of the swamp eel, *Synbranchus marmoratus*, an immunocytochemical analysis. Biocell 20:155–161

Vissio PG, Somoza G, Maggese MC, Paz DA, Strüsmann CA (1997) Structure and cell type distribution in the pituitary gland of pejerrey *Odontesthes bonariensis*. Fish Sci 63:64–68

van Zoest ID, Heijmen PS, Cruijsen PMJM, Jenk BG (1989) Dynamics of background adaptation in *Xenopus laevis*: role of catecholamines and melanophore stimulating hormone. Gen Comp Endocrinol 76:19–28. doi:10.1016/0016-6480(89)90028-2