Gill-derived glands in species of *Astyanax* (Teleostei: Characidae)

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Abstract

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The presence of a gill-derived gland is herein reported for the first time in males of species of *Astyanax* and related genera; they are described through histological cuts and SEM. The gill-derived glands described for the Characidae, when fully developed, present a similar structure in different species. The main external feature of gill-derived glands is the fusion of anteriormost gill filaments on the ventral branch of first gill arch. This fusion is caused by squamous stratified epithelial tissue that covers adjacent filaments, forming a series of chambers. In the region where the gill-derived gland develops, the secondary lamellae of the gill filaments are much reduced or completely atrophied being characterized by the presence of glandular cells forming nests.

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Introduction

The family Characidae is the most diversified among Neotropical fishes, with more than 1200 species (Reis *et al.* 2003). The great evolutionary success of the family may have been caused by many factors, among them chemical signals, which play a important role in the behaviour of some of these species (*e.g.* Bushmann and Burns 1994; Bushmann *et al.* 2002).

A caudal-fin gland has been known in species of the Characidae since early systematic works, and it was considered a diagnostic character for the subfamily Glandulocaudinae (Eigenmann 1914; Weitzman and Menezes 1998). That glandular tissue is contained in a cavity formed by skin and covered laterally by scales, becoming evident both in fresh and alcohol-preserved specimens. The caudal-fin gland is usually more developed in or restricted to males, whereas this gland is reduced or absent in females, depending on the species. Given such sexual dimorphism, this gland is considered to be associated with reproduction and especially with insemination, due to a high correlation between the last two features (Nelson 1964; Weitzman and Fink 1985; Burns *et al.* 1995). However, several species lacking a caudal-fin gland were discovered to have insemination (Burns *et al.* 1997; Burns and Weitzman 2005; Weitzman *et al.* 2005). In addition, other kinds of glandular tissues were discovered in non-glandulo-caudine species, and the importance of the chemical signals for reproductive behaviour, which were supposed to be only present in the Glandulocaudinae (*sensu* Weitzman and Menezes 1998), was also gradually being considered for many other characids.

The gill-derived glands were first described for the Characidae by Burns and Weitzman (1996) for males of *Corynopoma riisei* and later on for many other species of the family. When present, these glands have a relatively high conservative morphology among species. The gill-derived glands are formed by modified anterior gill filaments of the first arch, which are fused to each other forming chambers (Bushmann *et al.* 2002). Each chamber is limited anterior and posteriorly by gill filaments and laterally by a stratified squamous epithelial tissue (Oliveira *et al.* 2012), and it communicates to the exterior through openings in their distal region (Burns and Weitzman 1996; Bushmann *et al.* 2002). The lamellae of the modified filaments are usually shorter than those of the filaments retaining a fully developed respiratory function (Bushmann *et al.* 2002). The morphology of the glandular region of the first gill arch suggests that it completely lacks a respiratory function, given that the membrane covering the chambers is an obstacle to the flow of water and the filaments have very reduced lamellae and consequently a much more reduced surface for gas exchange. The gill-derived glands are present

only in sexually mature males and were not reported either in immature males or in females (Burns and Weitzman 1996; Bushmann *et al.* 2002; Azevedo *et al.* 2010; Oliveira *et al.* 2012). The development of such glands probably responds to an increase in testicular hormones and is apparently progressive with age, starting from the anteriormost filaments (Burns and Weitzman 1996). Gonçalves *et al.* (2005) found that the development of gill glands is correlated with testis maturation.

The fact that the gill-derived glands are present only in sexually mature males suggests that the released substance could be a pheromone involved in the reproduction, courtship and/or aggressive behaviour of males (Burns and Weitzman 1996; Bushmann *et al.* 2002).

The nature of the secretion and the function of the gill-derived glands have been experimentally tested by Shrestha *et al.* (2013) in *Aphyocharax anisitsi*. They demonstrated the presence of small metabolites, lipids and peptides/proteins in their gill-derived glands. Comparative analysis of gill tissues from fishes of different gender and age indicated that mature males had a unique protein in their gill glands.

Burns and Weitzman (1996) proposed that during the courtship of C. riisei, the males release the secretion from their gill glands by opening their opercles. The presence of pheromones associated with certain behaviours was noted in several Ostariophysi by Liley and Stacey (1983). The protein found by Shrestha *et al.* (2013) could correspond to the secretion mentioned by Burns and Weitzman (1996). However, both the exact nature of this protein in *A. anisitsi* and its variations in different species of Characidae are still unknown.

A gill-derived gland was reported for many members of the Stevardiinae and species of other clades, including the Aphyocharacinae and the Cheirodontinae (a complete list is given by Oliveira *et al.* 2012). Mirande (2009, 2010) analysed the presence or absence of gill-derived gland as a phylogenetic character within Characidae, coding it as present in several species of clades in which it had not been previously reported, as *Aphyocharacidium bolivianum* (Aphyoditeinae) and Phenacogaster tegatus (Characinae). However, Mirande (2009, 2010) coded this character based only on literature and macroscopic examination of alcohol-preserved specimens.

In the phylogenetic hypothesis by Mirande (2010), the presence of a gill-derived gland was optimized as a convergence in *P. tegatus* (the single species of *Phenacogaster* analysed in that work) and a clade composed of the Aphyocharacinae, Aphyoditeinae, Cheirodontinae and Stevardiinae, with several reversions in Aphyocharacinae and Stevardiinae. In the most comprehensive study to date about gill-derived glands in the Characidae, Oliveira *et al.* (2012) reported the presence of a gill-derived gland in Phenacogaster franciscoensis (they did not examine *P. tegatus*), many Stevardiinae and all the species of the Cheirodontinae examined by them. When present, the gill-derived glands of all the species exam-

ined by Oliveira *et al.* (2012) had the same structure; therefore, these authors proposed that the glands were homologous, appeared in the phylogeny of the Characidae only once and had multiple reversions to absence.

Oliveira *et al.* (2012) interpreted their observations on the phylogenetic hypotheses by Javonillo *et al.* (2010) and Oliveira *et al.* (2011) based on molecular data and Mirande (2010) based on morphology. In their interpretation based on hypotheses from molecular phylogenies, the gill-derived gland appeared in a clade composed of the Aphyocharacinae, (most) Characinae, Cheirodontinae, Stevardiinae and (some) Tetragonopterinae, which is the sister group of a 'clade C', which includes most species of the '*Astyanax* clade' of Mirande (2010), composed of most species of *Astyanax* plus *Markiana nigripinnis* and *Psellogrammus kennedyi* and some species of *Hyphessobrycon*. However, Oliveira *et al.* (2012) did not perform a character optimization and represented this gland as present in clades in which it is actually variable (Oliveira *et al.* 2012, figure 7). Thus, their interpretation of the evolution of this character based on the molecular trees is not necessarily supported by the actual distribution of the gill-derived gland in the Characidae if a parsimonious optimization of this character is done.

Oliveira *et al.* (2012) cited published observations (most of them macroscopic) about the absence of a gill-derived gland in *Astyanax courensis, A. goyanensis, A. jordanensis, A. laticeps, A. obscurus* and *A. utiariti; Ectrepopterus uruguayensis; Hasemania kalunga; Hemigrammus ora, H. tocantinsi, Hyphessobrycon melanostichos, H. notidanos* and *H. vinaceus;* and *Oligosarcus itau* (Carvalho and Bertaco 2006; Bertaco and Garutti 2007; Bertaco *et al.* 2007, 2010; Vera Alcaraz *et al.* 2009; Bertaco and Carvalho 2010; Bertaco and Lucena 2010; Carvalho *et al.* 2010; Jerep *et al.* 2011; Mirande *et al.* 2011; Malabarba *et al.* 2012). Based on that information, they considered the gill-derived glands as absent in the 'clade C' of the phylogenetic hypotheses of Javonillo *et al.* (2010) and Oliveira *et al.* (2011).

In this study, we survey and reveal the presence of a gill-derived gland in species of the *Astyanax* clade (*sensu* Mirande 2010), based on histological examination of mature males.

Materials and Methods

Work material – male and female adult specimens – is deposited in the ichthyological collection of the Fundación Miguel Lillo (CI-FML). The deposited samples were fixed in 10% formaldehyde and subsequently preserved in 70% ethanol.

The gill skeleton was dissected from studied specimens. The left arch was used for histology and the right arch for scanning electron microscopy (SEM).

For the histological techniques, the material was decalcified with 10% formic acid for a week, then dehydrated in ethanol series and included in paraplast. It was sectioned parallel to the ceratohyal (sagittal cuts) with cuts every 6–7 μm. The

staining of most samples was carried out with haematoxylin–eosin.

Adult specimens of sexually mature species of the Astyanax clade were selected for this analysis. Several individuals were macroscopically analysed to ascertain the presence of gill-derived glands; the material prepared for histological analysis was as follows:

Astyanax abramis. CI-FML 5422. Argentina, Corrientes, Ituzaingo, río Paraná. Río Paraná basin. 1 male and 1 female. CI-FML 5040. Argentina, Salta, río Juramento. Río Juramento basin. 2 males. CI-FML 5848. Argentina, Salta, río Arenales. Río Juramento basin. 1 male. *Astyanax asuncionensis*. CI-FML 3299. Argentina, Salta, Orán, El Oculto, río Blanco. río Bermejo basin. 2 males and 1 female. CI-FML 3912. Argentina, Tucumán, Monteros, río Mandolo. Río Salí-Dulce basin. 1 male. CI-FML 5802. Argentina, Tucumán, Simoca, arroyo Los Perez. Río Salí-Dulce basin. 2 males and 1 female. *Astyanax chico*. CI-FML 5806. Argentina, Salta, General San Martin, near Campo Largo. Río Bermejo basin. 1 male and 1 female. CI-FML 3263. Argentina, Salta, El Oculto, La Bambú. Río Bermejo basin. 1 male. *Astyanax eigenmanniorum*. CI-FML 5468. Brazil, Rio Grande do Sul, Lagoa Bacupan. 1 male. *Astyanax* cf. *eigenmanniorum* 1. CI-FML 3037. Argentina, Tucumán, Tafí Viejo, río Loro. Río Sali-Dulce basin. 4 males and 1 female. *Astyanax* cf. eigenmanniorum 2. CI-FML 3914. Argentina, Santiago del Estero, Dique Los Quiroga, río Dulce. Río Salí-Dulce basin. 3 males and 1 female. *Astyanax endy*. CI-FML 3357. Argentina, Salta, Orán, arroyo El Oculto. Río Bermejo basin. 1 male. CI-FML 3384. Argentina, Salta, Orán, río Anta Muerta. Río Bermejo basin. 1 male. CI-FML 3326. Argentina, Salta, Orán, arroyo El Oculto. Río Bermejo basin. 1 female. *Astyanax leonidas*. CI-FML 5470. Argentina, Misiones, Embalse Urugua-í. Río Paraná basin. 4 males and 1 female. *Astyanax lineatus*. CI-FML 5469. Argentina, Salta, río Arenales. Río Juramento basin. 2 males and 1 female. *Astyanax ojiara*. CI-FML 5471, 2 males, Argentina, Misiones, Arroyo Yabotí, Río Uruguay. Río Uruguay basin. *Astyanax paranae*. CI-FML 5467. Brazil, Riberão, Paraiso do Norte, Rio Ivai. Río Paraná basin. 2 males. *Astyanax puka*. CI-FML 5371. Argentina, Tucumán, Simoca, arroyo Pampa-Mayo. Río Sali-Dulce basin. 1 male. CI-FML 5803. Argentina, Tucumán, Simoca, arroyo Pampa-Mayo. Río Sali-Dulce basin. 1 male and 1 female. *Astyanax* cf. *rutilus*. CI-FML 5805. Argentina, Tucumán, Trancas, Embalse Celestino Gelsi. Río Salí-Dulce basin. 2 males and 1 female. *Astyanax troya*. CI-FML 5466. Argentina, Misiones, Aristóbulo del Valle, PP Salto Encantado, río Cuña Pirú. Río Paraná basin. 2 males and 1 female. CI-FML 3920. Argentina, Misiones, Aristóbulo del Valle, PP Salto Encantado, río Cuña Pirú. Río Paraná basin. 1 male. *Hyphessobrycon anisitsi*. CI-FML 3926. Argentina, Santa Fe, Garay, Helvecia, río San Javier. Río Paraná basin. 4 males and 1 female. *Markiana nigripinnis*. CI-FML 2644. Argentina, Cañada El Hogar, Misión La Paz, Salta, río Pilcomayo. Río Pilcomayo basin. 2 males and 1 female. *Psellogrammus*

kennedyi. CI-FML 3946. Paraguay, Alto Paraguay, Bahía Negra, Fortín Patria, río Paraguay. Río Paraguay basin. 2 males and 1 female.

Results

Gill-derived glands in species of *Astyanax* are herein recorded for the first time. Their structure in the examined species is similar to that described for other characids, where a fusion of the anteriormost filaments was observed in previous studies (Gonçalves *et al.* 2005; Bührnheim and Malabarba 2006, 2007; Mirande 2009, 2010; Azevedo *et al.* 2010; Jerep and Malabarba 2011; Oliveira *et al.* 2012).

The anteriormost filaments are strongly modified for a glandular function, apparently losing their primary respiratory function. These filaments bear packs of columnar gland cells situated between successive secondary lamellae, which are
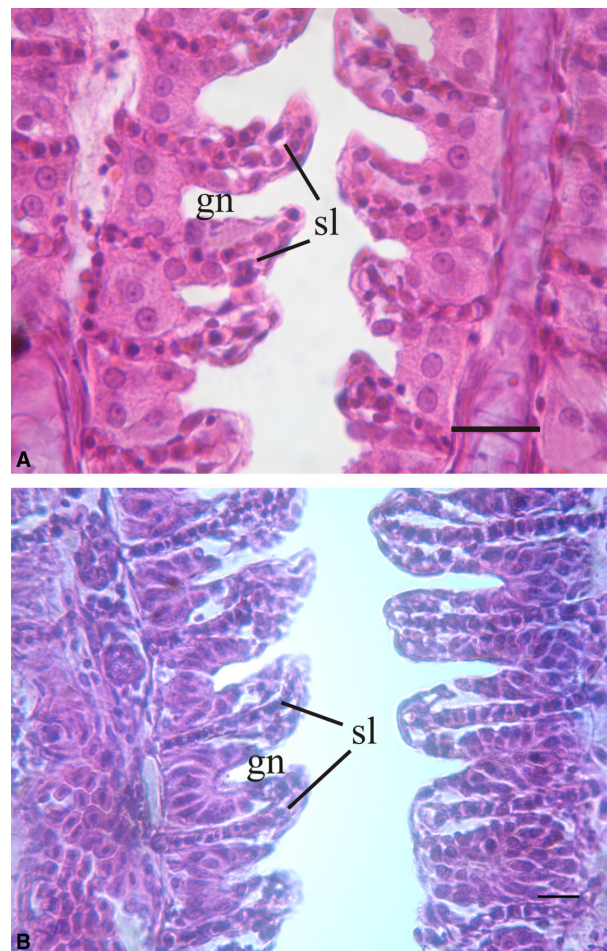

Fig. 1—Histological sections through first left gill arches, lateral view. Glandular nests between the secondary lamella. Stained with haematoxylin–eosin. (A) *Astyanax puka* (scale bar: 10 µm). (B) *A. paranae* (scale bar: 20 µm). sl, gill secondary lamellae; gn, glandular nest.

herein called 'glandular nests' (Fig. 1). The modified filaments are covered by stratified squamous epithelial tissue, forming a glandular capsule composed of contiguous chambers situated between each pair of filaments. These capsules are easily recognizable macroscopically. A fully developed gill-derived gland, as described here, was observed in males of *Astyanax eigenmanniorum* (Figs 2A and 4A), *A. leonidas* (Figs 2B, 3A and 4B), *A. ojiara* (Figs 2C, 3B and 4C) and *A. puka* (Figs 2D, 3C and 4D).

Some species of Astyanax presented only partially formed gill-derived glands, with just two or three filaments fused to each other forming chambers, separated by unmodified respiratory filaments. Fused filaments have their halves oriented outside the chamber without modifications from its primary respiratory function, while the spaces between the lamellae oriented to the chamber are filled with glandular cells. This condition was herein observed in males of *A.* cf. *eigenmanniorum* 1, *A.* cf. *eigenmanniorum* 2 (Fig. 4E), *A. endy* (Figs 2E and 4F), *A. troya* (Fig. 2F) and *H. anisitsi* (Fig. 5A). It is not clear, however, whether this kind of gill-derived gland is the final stage of development for these species, or whether it rep-

resents ontogenetically intermediate states relative to the condition described above in which the capsules are fully developed.

In other species, we also found glandular cells but not fusion on the gill filaments, or if present, the fusion is restricted to the base of the anteriormost ones and not occurring along the complete extension of the filaments. This was observed in males of *Astyanax asuncionensis* (Fig. 5B), *A. chico, A. lineatus, A.* cf. *rutilus* and *A. paranae* (Figs 1B and 5C). In A. chico, glandular cells were also observed on filaments attached to the first epibranchial. However, it is still unknown whether a complete gill-derived gland is developed in some males of these species. Even in species with the least modified gill-derived glands, the glandular nests are present only in males.

In some species, we did not find any glandular cells in the gill filaments of mature males, and their condition is identical to that of females, but the examination of more specimens is necessary to better corroborate the absence of the gill-derived gland in these species. This condition was herein observed in *A. abramis, M. nigripinnis* and *P. kennedy.*
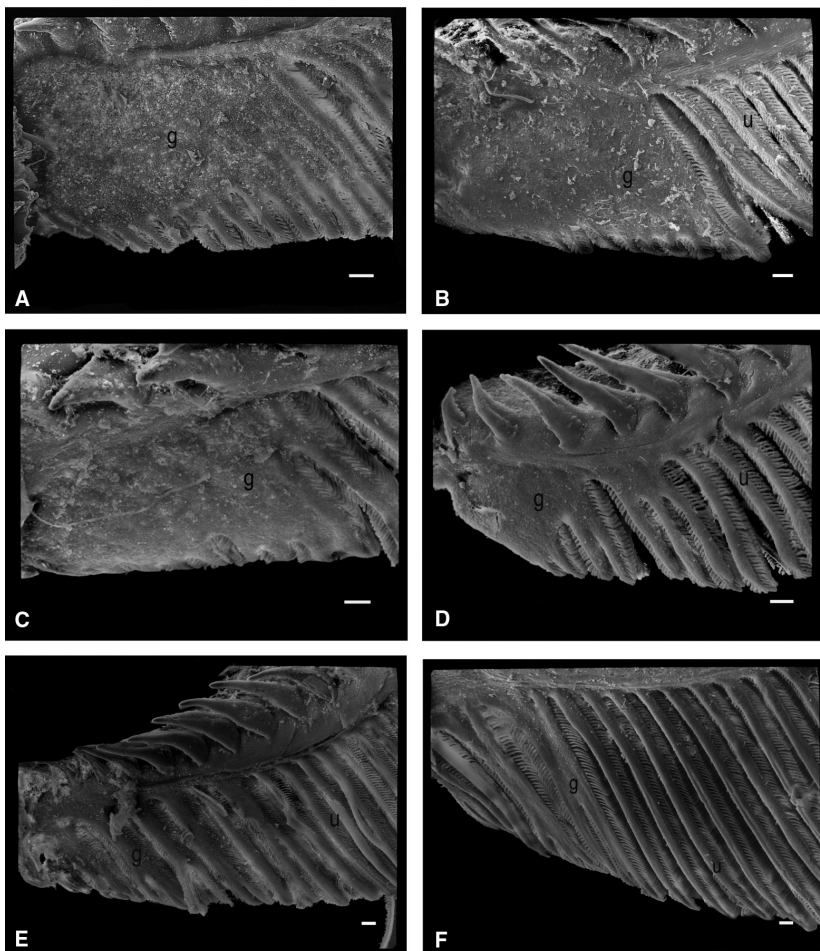

Fig. 2—Scanning electron micrographs (SEMs) of first right gill arch, lateral view, anterior to left of males of *Astyanax*. (A) *A. eigenmanniorum*; (B) *A. leonidas*; (C) *A. ojiara*; (D) *A. puka*; (E) *A. endy*; and (F) *A. troya*. Scale bars: 100 μm. g, gill glands; u, unmodificated filaments.
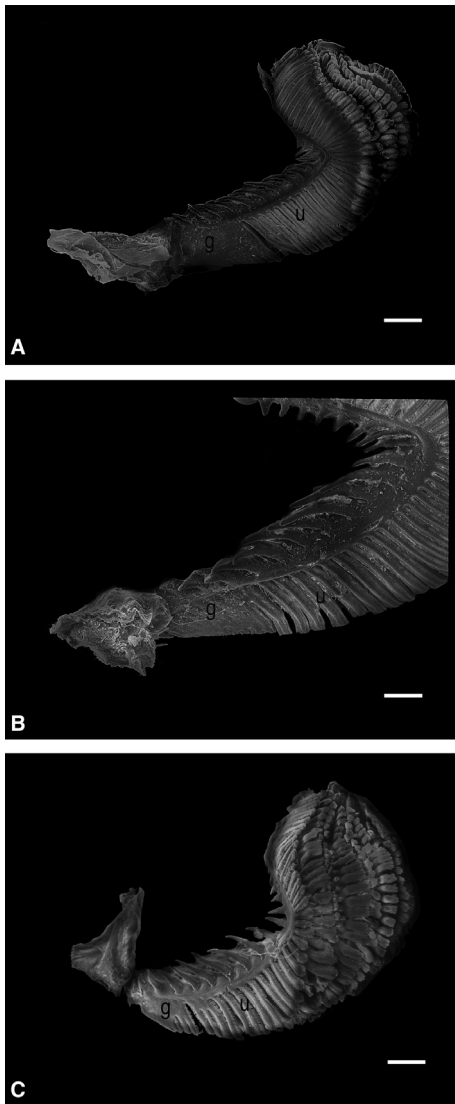
Fig. 3—Scanning electron micrographs (SEMs) of first right gill arch, lateral view, anterior to left of males of *Astyanax*. (A) *A. leonidas* (scale bar: 1000 μm); (B) *A. ojiara* (scale bar: 500 μm); and C: *A. puka* (scale bar: 500 μm). g, gill glands; u, unmodificated filaments.

Discussion

The presence of gill-derived glands is herein reported for the first time in species of *Astyanax*. The gill-derived glands present in members of the Characidae a conservative structural morphology when fully developed. The main external feature of the gill-derived glands is the fusion of anteriormost gill filaments of the ventral branch of first gill arch. This fusion is due to a squamous stratified epithelial tissue that covers adjacent filaments, forming chambers (Bushmann *et al.* 2002). In the region where the gill-derived gland develops, the secondary lamellae of the gill filaments are greatly reduced or completely atrophied with the presence of glandular cells forming nests

opening between adjacent lamellae (Burns and Weitzman 1996; Bushmann *et al.* 2002; Weitzman *et al.* 2005; Azevedo *et al.* 2010; Oliveira *et al.* 2012).

The morphological conservativeness of the gill-derived glands led Oliveira *et al.* (2012) to propose the homology and unique origin of this structure in Characidae. They compared the distribution of this character on morphological- and molecular-based phylogenetic analyses of the family (Javonillo *et al.* 2010; Mirande 2010; Oliveira *et al.* 2011). However, they mapped the presence of gill-derived glands in clades where the glands have variable occurrence (Aphyocharacinae, Characinae and Stevardiinae), while this character is probably most parsimoniously optimized as absent at the base of some of these clades if the actual distribution of states within each node is considered.

Oliveira *et al.* (2012) proposed a single origin of a gill-derived gland as a putative synapomorphy of the lineage composed of the clades A (Stevardiinae) and B (Aphyocharacinae, Aphyoditeinae, Characinae and Cheirodontinae) of Javonillo *et al.* (2010) and Oliveira *et al.* (2011), with reversions in Tetragonopterus and in a clade composed of Exodon and Roeboexodon. Oliveira *et al.* (2012) optimized the gill-derived gland as absent in the clade C (composed mainly of the Tetragonopterinae and the *Astyanax* clade of Mirande 2010) of the molecular-based phylogenetic hypotheses of Javonillo *et al.* (2010) and Oliveira *et al.* (2011).

According to the results herein obtained, if the presence of the gill-derived glandular tissue had a single origin in the phylogeny of the Characidae, it should be a synapomorphy of a large clade composed of all the species lacking a supraorbital bone. This conclusion is reachable projecting the distribution of this feature to all the clade C of Javonillo *et al.* (2010) and Oliveira *et al.* (2011), in the same way that Oliveira *et al.* (2012) proposed the presence of this gland to the common ancestor of the Characinae from observations made in *Phenacogaster*.

Secondary sexual characters are known to be present in most species of the Characidae, being the fin hooks the most widely studied (*e.g.* Wiley and Collette 1970; Azpelicueta and García 2000; Lima and Sousa 2009). Some species of Characidae also have other evident features, such as a caudal-fin gland, elongation of fins, a urogenital papilla, presence of breeding tubercles, expansion of procurrent caudal-fin rays, modifications in the form and orientation of pelvic bones and fins, and/or a well-developed gill-derived gland (*e.g.* Wiley and Collette 1970; Malabarba 1998; Weitzman and Menezes 1998; Mirande *et al.* 2004; Zanata and Akama 2004; Melo and Buckup 2006). The single known secondary sexual character reported in *Astyanax* is the presence of bony hooks on fins, usually in the anal and pelvic fins, and the elongation of the dorsal fin in some species (*e.g.* Azpelicueta *et al.* 2002; Melo and Buckup 2006). The presence of secondary sexual glands was originally found in species having insemination or unusual secondary sexual characters (*e.g. C. riisei*; Burns and Weitzman 1996), and subsequently, these structures were
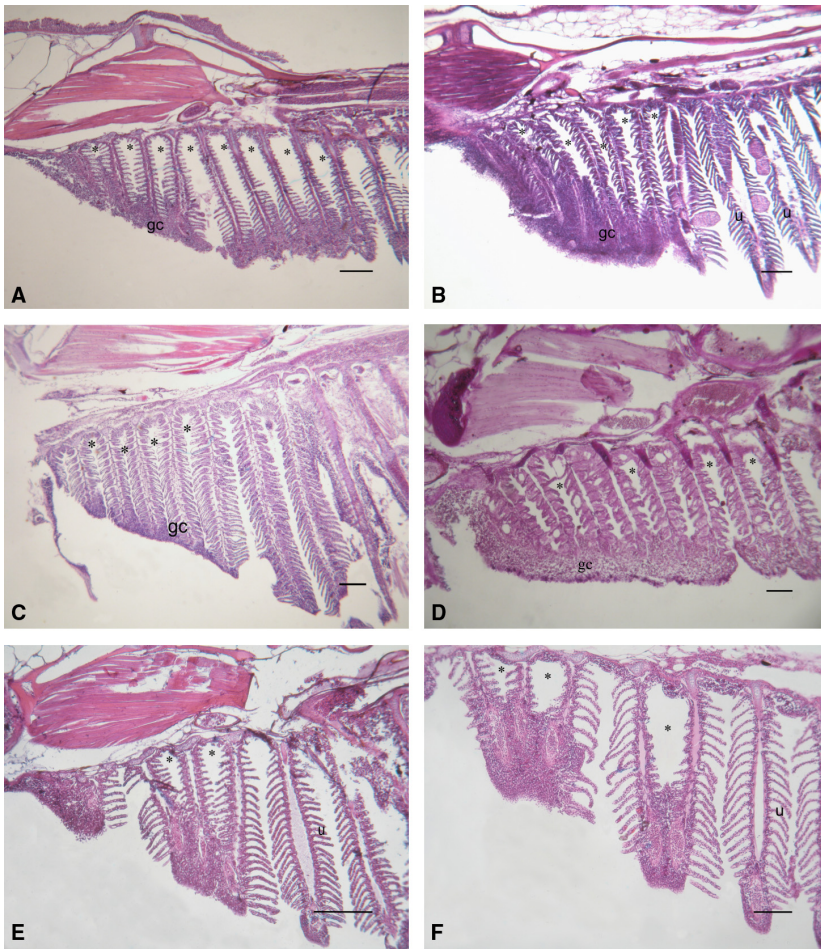
Fig. 4—Sagittal histological sections through first left gill arches, lateral view, anterior to left of males of *Astyanax* stained with haematoxylin–eosin. (A) *A. eigenmanniorum* (scale bar: 200 μm); (B) *A. leonidas* (scale bar: 100 μm); (C) *A. ojiara* (scale bar: 100 μm); (D) *A. puka* (scale bar: 100 μm); (E) *A.* cf. *eigenmanniorum* 2 (scale bar: 200 μm); and (F) *A. endy* (scale bar: 100 μm). *lumen of gill gland chamber; u, unmodificated filaments; gc, gill gland cover.
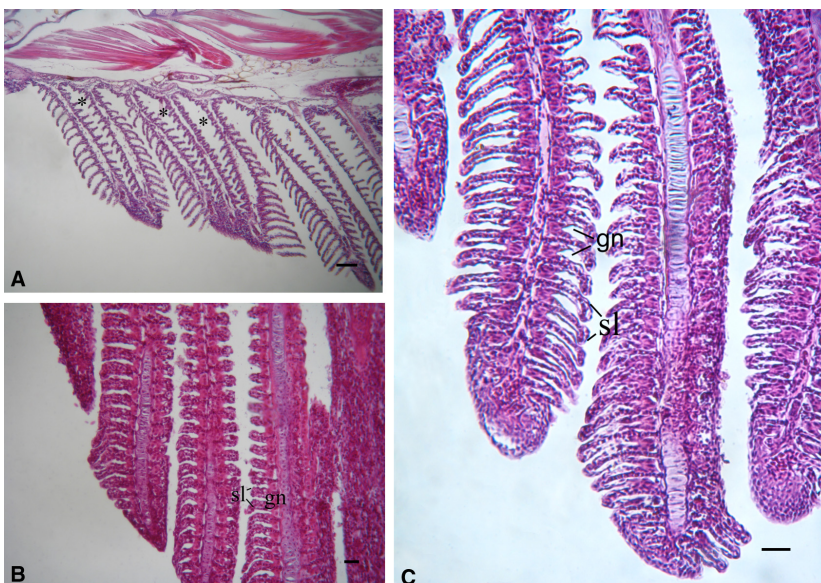

Fig. 5—Histological sections through anterior left gill arches of males, lateral view, anterior to left stained with haematoxylin–eosin. (A) *Hyphessobrycon anisitsi* (scale bar: 100 μm); (B) *Astyanax asuncionensis* (scale bar: 30 μm); and (C) *A. paranae* (scale bar: 30 μm). *lumen of gill gland chamber. sl, gill secondary lamellae; gn, glandular nest.

reported in some species with relatively less sexual dimorphism (*e.g. Odontostilbe fugitiva*; Oliveira *et al.* 2012).

This article demonstrates the presence of gill-derived glands in some species of the *Astyanax* clade as an additional secondary sexual character. The evidence herein presented shows that the gill-derived glands have a wider distribution among characids.

Acknowledgements

References

Azevedo, M. A, Malabarba, L. R. and Burns, J. R. 2010. Reproductive biology and development of gill glands in the inseminating characid, *Macropsobrycon uruguayanae* Eigenmann, 1915 (Cheirodontinae: Compsurini). *Neotropical Ichthyology* **8**: 87–96.

Azpelicueta, M. and García, J.O. 2000. A new species of *Astyanax* (Characiformes: Characidae) from Uruguay River Basin in Argentina, with remarks on hook presence in Characidae. *Revue suisse De Zoologie* **107**: 245–257.

Azpelicueta, M. M, Casciotta, J. R. and Almirón, A. E. 2002. Two new species of the genus *Astyanax* (Characiformes, Characidae) from the Paraná River basin in Argentina. *Revue suisse de Zoologie* **109**: 243–259.

Bertaco, V. A. and Carvalho, F. R. 2010. New species of *Hasemania* (Characiformes: Characidae) from Central Brazil, with comments on the endemism of upper rio Tocantins basin, Goiás State. *Neotropical Ichthyology* **8**: 27–32.

Bertaco, V. A. and Garutti, V. 2007. New *Astyanax* from the upper rio Tapajós drainage, Central Brazil (Characiformes: Characidae). *Neotropical Ichthyology* **5**: 25–30.

Bertaco, V. A. and Lucena, C. A. S. 2010. Redescription of *Astyanax obscurus* (Hensel, 1870) and *A. laticeps* (Cope, 1894) (Teleostei: Characidae): Two valid freshwater species originally described from rivers of Southern Brazil. *Neotropical Ichthyology* **8**: 7–20.

Bertaco, V. A, Malabarba, L. R. and Dergam, J. A. 2007. New *Hyphessobrycon* from the upper rio Pardo drainage in eastern Brazil (Teleostei: Characiformes: Characidae). *Neotropical Ichthyology* **5**: 245–249.

Bertaco, V. A, Carvalho, F. R. and Jerep, F. C. 2010. *Astyanax goyanensis* (Miranda-Ribeiro, 1944), new combination and *Astyanax courensis*, new species (Ostariophysi: Characiformes): Two Characidae from the upper rio Tocantins basin, Central Brazil. *Neotropical Ichthyology* **8**: 265–275.

Bührnheim, C. M. and Malabarba, L. R. 2006. Redescription of the type species of *Odontostilbe* Cope, 1870 (Teleostei: Characidae:

Cheirodontinae), and description of three new species from the Amazon basin. *Neotropical Ichthyology* **4**: 167–196.

Bührnheim, C. M. and Malabarba, L. R. 2007. Redescription of *Odontostilbe pulchra* (Gill, 1858) (Teleostei: Characidae: Cheirodontinae), and description of two new species from the río Orinoco basin. *Neotropical Ichthyology* **5**: 1–20.

Burns, J. R. and Weitzman, S. H. 1996. Novel gill-derived gland in the male swordtail characin, *Corynopoma riisei* (Teleostei: Characidae: Glandulocaudinae). *Copeia* **1996**: 627–633.

Burns, J. R. and Weitzman, S. H. 2005. Insemination in ostariophysan fishes. In: Uribe, M. C. and Grier, H. J. (Eds): Viviparous Fishes, vol **11**, pp. 107–134, New Life Publishers, Florida.

Burns, J. R, Weitzman, S. H, Grier, H. J. and Menezes, N. A. 1995. Internal fertilization, testis and sperm morphology in glandulocaudine fishes (Teleostei: Characidae: Glandulocaudinae). *Journal of Morphology* **224**: 131–145.

Burns, J. R, Weitzman, S. H. and Malabarba, L. R. 1997. Insemination in eight species of Cheirodontine fishes (Teleostei: Characidae: Cheirodontinae). *Copeia* **1997**: 433–438.

Bushmann, P. J and Burns, J. R. 1994. Social control of male sexual maturation in the swordtail characin, *Corynopoma riisei*. *Journal of Fish Biology* **44**: 263–272.

Bushmann, P. J, Burns, J. R. and Weitzman, S. H. 2002. Gill-derived glands in glandulocaudine fishes (Teleostei: Characidae: Glandulocaudinae). *Journal of Morphology* **253**: 187–195.

Carvalho, T. P. and Bertaco, V. A. 2006. Two new species of *Hyphessobrycon* (Teleostei: Characidae) from upper rio Tapajós basin on Chapada dos Parecis, Central Brazil. *Neotropical Ichthyology* **4**: 301–308.

Carvalho, F. R, Bertaco, V. A. and Jerep, F. C. 2010. *Hemigrammus tocantinsi*: A new species from the upper rio Tocantins basin, Central Brazil (Characiformes: Characidae). *Neotropical Ichthyology* **8**: 247–254.

Eigenmann, C. H. 1914. Some results from studies of South American fishes. *Indiana University Studies* **20**: 19–43.

Gonçalves, T. K, Azevedo, M. A, Malabarba, L. R. and Fialho, C. B. 2005. Reproductive biology and development of sexually dimorphic structures in *Aphyocharax anisitsi* (Ostariophysi: Characidae). *Neotropical Ichthyology* **3**: 433–438.

Javonillo, R, Malabarba, L. R, Weitzman, S. H and Burns, J. R. 2010. Relationships among major lineages of characid fishes (Teleostei: Ostariophysi: Characiformes), based on molecular sequence data. *Molecular Phylogenetics and Evolution* **54**: 498–511.

Jerep, F. C. and Malabarba, L. R. 2011. Revision of the genus *Macropsobrycon* Eigenmann, 1915 (Characidae: Cheirodontinae: Compsurini). *Neotropical Ichthyology* **9**: 299–312.

Jerep, F. C, Carvalho, F. C. and Bertaco, V. A. 2011. Geographic distribution of *Hemigrammus ora* (Ostariophysi: Characiformes: Characidae) in the Amazon basin, Brazil. *Zoologia (Sociedade Brasileira de Zoologia)* **28**: 545–550.

Liley, N. R. and Stacey, N. E. 1983. Hormones, pheromones, and reproductive behavior in fish. In: Hoar, W. S, Randall, D. J. and Donaldson, E.M. (Eds): Fish Physiology, vol. **9B**, pp. 1–63. Academic Press, New York, NY.

Lima, T. and Sousa, L. M. 2009. A new species of *Hemigrammus* from the upper rio Negro basin, Brazil, with comments on the presence and arrangement of anal-fin hooks in Hemigrammus and related genera (Ostariophysi: Characiformes: Characidae). *Aqua, International Journal of Ichthyology* **15**: 153–168.

Malabarba, L. R. 1998. Monophyly of the Cheirodontinae, characters and major clades (Ostariophysi: Characidae). In: Malabarba, L. R, Reis, R. E, Vari, R. P, Lucena, Z. M. S. and Lucena, C. A. S.

(Eds): Phylogeny and Classification of Neotropical Fishes, pp. 193–233. Edipucrs, Porto Alegre.

Malabarba, L. R, Bertaco, V. A, Carvalho, F. R. and Litz, T. O. 2012. Revalidation of the genus *Ectrepopterus* Fowler (Teleostei: Characiformes), with the redescription of its type species, *E. uruguayensis. Zootaxa* **3204**: 47–60.

Melo, F. A. G. and Buckup, P. A. 2006. *Astyanax henseli*, a new name for *Tetragonopterus aeneus* Hensel, 1870 from southern Brazil (Teleostei: Characiformes). *Neotropical Ichthyology* **4**: 45–52.

Mirande, J. M. 2009. Weighted parsimony phylogeny of the family Characidae (Teleostei: Characiformes). *Cladistics* **25**: 574–613.

Mirande, J. M. 2010. Phylogeny of the family Characidae (Teleostei: Characiformes): From characters to taxonomy. *Neotropical Ichthyology* **8**: 385–568.

Mirande, J. M, Aguilera, G. and Azpelicueta, M. M. 2004. A new genus and species of small characid (Ostariophysi, Characidae) from the upper río Bermejo basin, northwestern Argentina. *Revue suisse de Zoologie* **111**: 715–728.

Mirande, J. M, Aguilera, G. and Azpelicueta, M. M. 2011. A threatened new species of *Oligosarcus* and its phylogenetic relationships, with comments on *Astyanacinus* (Teleostei: Characidae). *Zootaxa* **2994**: 1–20.

Nelson, K. 1964. Behavior and morphology in the glandulocaudine fishes (Ostariophysi, Characidae). *University of California Publications in Zoology* **75**: 59–152.

Oliveira, C, Avelino, G. S, Abe, K. T, Mariguela, T. C, Benine, R. C, Ortí, G, Vari, R. P. and Castro, R. M. C. 2011. Phylogenetic relationships within the speciose family Characidae (Teleostei: Ostariophysi: Characiformes) based on multilocus analysis and extensive ingroup sampling. *BMC Evolutionary Biology* **11**: 275.

Oliveira, C. L. C, Malabarba, L. R. and Burns, J. R. 2012. Comparative morphology of gill glands in externally fertilizing and inseminating species of cheirodontine fishes, with implications on the phylogeny of the family Characidae (Actinopterygii: Characiformes). *Neotropical Ichthyology* **10**: 349–360.

Reis, R. E., Kullander, S. O. and Ferraris, C. J. Jr 2003. Check List of the Freshwater Fishes of South and Central America. Edipucrs, Porto Alegre, pp.729.

Shrestha, B, Javonillo, R, Burns, J. R, Pirger, Z. and Vertes, A. 2013. Comparative local analysis of metabolites, lipids and proteins in intact fish tissues by LAESI mass spectrometry. *Analyst* **138**: 3444–3449.

Vera Alcaraz, H. S, Pavanelli, C. S. and Bertaco, V. A. 2009. *Astyanax jordanensis* (Ostariophysi: Characidae), a new species from the rio Iguaçu basin, Paraná, Brazil. *Neotropical Ichthyology* **7**: 185–190.

Weitzman, S. H. and Fink, S. V. 1985. Xenurobryconin phylogeny and putative pheromone pumps in glandulocaudine fishes (Teleostei: Characidae). *Smithsonian Contributions to Zoology* **421**: 1–121.

Weitzman, S. H. and Menezes, N. A. 1998. Relationships of the tribes and genera of the Glandulocaudinae (Ostariophysi: Characiformes: Characidae) with a description of a new genus, *Chrysobrycon*. In: Malabarba, L. R, Reis, R. E, Vari, R. P, Lucena, Z. M. S. and Lucena, C. A. S. (Eds): Phylogeny and Classification of Neotropical Fishes, pp. 171–192. Edipucrs, Porto Alegre.

Weitzman, S. H, Menezes, N. A, Evers, H. G. and Burns, J. R. 2005. Putative relationships among inseminating and externally fertilizing characids, with description of a new genus and species of Brazilian inseminating fish bearing an anal-fin gland in males (Characiformes: Characidae). *Neotropical Ichthyology* **3**: 329–360.

Wiley, M. L. and Collette, B. B. 1970. Breeding tubercles and contact organs in fishes: Their occurrence, structure, and significance. *Bulletin of the American Museum of Natural History* **143**: 143–216.

Zanata, A. M. and Akama, A. 2004. *Myxiops aphos*, new characid genus and species (Characiformes: Characidae) from the rio Lençóis, Bahia, Brazil. *Neotropical Ichthyology* **2**: 45–54.