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A new species of *Astyanax* (Characiformes, Characidae) from Paraná river basin in Argentina

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Resumen. Se describe *Astyanax tupi sp. n*. colectada en los arroyos Cuñapirú y Cuñapirú Chico, afluentes del río Paraná en la provincia de Misiones, noreste de Argentina. Una combinación de caracteres diferencia a la nueva especie de sus congéneres: aletas pectorales largas, siempre sobrepasando el origen de la aleta pélvica; origen de la aleta anal a nivel de una vertical a través del quinto o sexto radio dorsal; una mancha supraopercular y dos manchas humerales en los flancos; 2-3 dientes maxilares; ojo (10.2-11.9 % LE); longitud maxilar (100.0-136.6 % en ancho interorbitario); 35-38 escamas perforadas en la línea lateral, 24-27 radios anales ramificados y cuerpo alto (36.9-42.6 % LE).

P**alabras claves**: *Characiformes*, *Characidae*, *Astyanax*, *río Paraná.*

Abstract. *Astyanax tupi* n. sp. is described from the streams Cuñapirú Chico and Cuñapirú, draining in Paraná river in the province of Misiones, Argentinean northeast. A combination of characters differentiates the new species from other congeners: long pectoral fin always surpassing the pelvic-fin origin; origin of anal fin at level of a vertical through fifth or sixth branched dorsal-fin rays; one supraopercular and two humeral spots on flanks; 2-3 maxillary teeth; (10.2-11.9 % SL); maxillary length (100.0-136.6 % interorbital width); 35-38 perforated scales in lateral line; 24-27 branched anal fin rays, and deep body (36.9-42.6 % SL).

Key-words: *Characiformes*, *Characidae*, *Astyanax*, *Paraná river.*

Introduction

The genus *Astyanax* Baird & Girard 1854 was revised by Eigenmann (1921, 1927) and subsequently by Géry (1977). In the last years, several authors published descriptions of new

species of *Astyanax* (among others, Garutti & Britski, 1997, 2000; Bertaco & Malabarba, 2001). In the río de la Plata basin, more than twenty nominal species of *Astyanax* have been recorded, although the records of some species are doubtful. The Sierras de Misiones, de Imán, and de la Victoria divide the province of Misiones, -in the northeast of Argentina- in three different slopes which carry their waters to the rivers Iguazú, Paraná and Uruguay respectively. Many collecting trips to the streams of all three basins allowed us to describe several species of *Astyanax* in the last few years (Azpelicueta & García, 2000; Azpelicueta *et al.*, 2002a,b; Almirón *et al.*, 2002; Casciotta *et al.*, in press). The objective of the present paper is to describe an addtional new species of the genus from the arroyos Cuñapirú and Cuñapirú Chico, tributaries of the río Paraná, in the province of Misiones.

Material and Methods

Measurements are straight distances taken with caliper to the nearest 0.1 mm. Measurements are expressed as percenttages of SL or the length indicated. Peduncle length was measured from the last anal fin ray insertion to the hypural joint. Mann Whitney test (significance $p<0.001$), principal component (PCA) and discriminant (DA) analyses were performed upon $\log_{10}$ transformed morphometric values. A correlation matrix without axis rotation was used, whereby the first component is a value of the SL and all measurements related to it. The second principal component mainly contains variables that are not correlated with SL in the complex sample; therefore, it is useful for differentiation among species. Stepwise DA was applied to known variables with high discriminant values; the DA shows the best ratio for differentiation among species. The specimens examined in this study were cleared and counterstained (C&S) following Taylor & Van Dyke (1985). Vertebrae counts include the Weber apparatus and the complex centrum as one element. Material is deposited in the following collections: Academy of Natural Sciences of Philadelphia, Philadelphia (ANSP); Asociación Ictiológica, La Plata (AI); Facultad de Ciencias Naturales y Museo de La Plata, La Plata (MLP); Fundación Miguel Lillo, Tucumán (CI-FML); Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires (MACN-Ict), and Museum d'histoire naturelle de Gèneve, Gèneve (MHNG).

Comparative material

Astyanax abramis (Jenyns, 1842): MLP 9427, 2 specimens, 102.0-113.0 mm SL, Argentina, Misiones, río Paraná. *Astyanax asuncionensis* Géry, 1972: MLP 8660, 5, 43.6-61.4 mm, Argentina, Santiago del Estero, Bañado de Añatuya. *Astyanax eigenmanniorum* (Cope, 1894): ANSP 21627-28, 2 paratypes, 42.5-49.4 mm, Brasil, Rio Grande do Sul; MLP 5202, 5, 56.5-68.5 mm, Argentina, Córdoba, río Primero in front of Capilla de los Remedios; MLP 9160, 6, 36.8-80.2 mm, Argentina, Buenos Aires, Los Talas. *Astyanax cf. fasciatus* (Cuvier, 1819): MLP 8668, 4, 61.0-67.7 mm, Argentina, Santiago del Estero, Bañado de Figueroa. MLP 8798, 17, 28.8-39.6 mm, Argentina, Formosa, highway from Formosa to Clorinda. *Astyanax ita* Almirón *et al.*, 2002: MLP 9599, holotype, 64.0 mm, Argentina, Misiones, Iguazú basin, arroyo Tateto. *Astyanax* sp. A (Mirande *et al.*, in press): CI-FML 3400, holotype, 44.3 mm, Argentina, Salta, río Bermejo basin, arroyo El Oculto. *Astyanax leonidas* Azpelicueta *et al.*, 2002: MLP 9580, holotype male, 45.6 mm, Argentina, Misiones, río Paraná basin, headwaters of arroyo Urugua-í. *Astyanax lineatus* (Perugia, 1891): CI-FML 3272, 3, 35.3-72.1 mm, Argentina, Salta, Orán, La Bambú, río Bermejo basin, río Blanco. .*Astyanax ojiara* Azpelicueta & García 2000: MLP 9470, holotype male, 50.5 mm, Argentina, Misiones, arroyo Benítez, headwaters of arroyo Yabotí-Miní, tributary of río Uruguay. *Astyanax paris* Azpelicueta *et al.*, 2002: MLP 9584, holotype, Argentina, Misiones, río Uruguay basin, arroyo Fortaleza. *Astyanax* sp. B (Casciotta *et al.*, in press): MACN-Ict 8543, holotype, 52.0 mm, Argentina, Corrientes, Esteros del Iberá, Laguna Iberá, Lobo-Cua. *Astyanax* sp. C (Casciotta *et al.*, in press): MLP 9603, holotype**,** 63.0 mm, Argentina, Misiones, río Uruguay basin, headwater

of arroyo Once Vueltas; MHNG 2639.47, 9 paratypes, 64.3-90.3 mm; same collecting data as holotype. *Astyanax troya* Azpelicueta *et al.*, 2002: MACN-Ict 8310, holotype, 73.8 mm, Argentina, Misiones, río Paraná basin, arroyo Cuñapirú Chico.

Results

Astyanax tupi, new species
Figs. 1-7, tables 1-6

Holotype

MACN-Ict 8646, 70.1 mm SL, Argentina, province of Misiones, arroyo Cuñapirú in Balneario de Aristóbulo del Valle, coll. U. Pardiñas, September 1997 (Fig. 1).

Paratypes

MHNG 2642.091, 6 ex., 61.0-72.6 mm SL, collected with the holotype. AI 128, 4 ex., 60.8-70.0 mm, Argentina, province of Misiones, arroyo Cuñapirú Chico, coll. E. D. Rodríguez, August 1993. ANSP 179251, 4 ex., 66.6-73.2 mm SL, collected with the holotype.

Diagnosis

Astyanax tupi is distinguished from other species of the genus by a combination of characters: long pectoral fins always surpassing pelvic-fin origin; anal fin origin at level of a vertical through fifth or sixth dorsal fin rays; one supraopercular and two humeral spots on flanks,; two or three maxillary teeth; 35-38 perforated scales in the lateral series, and 24-27 branched anal fin rays. Also, the deep body (36.9-42.6 % SL), large eye (10.2-11.9 % SL), maxillary length (100.0-136.6 % interorbital width), and presence of 24-25 gill rakers on first branchial arch differentiate the new species from most of its congeneres.

Description

Morphometrics of holotype and 14 paratypes are presented in table 1. *Astyanax* with deep body (Fig. 1), maximum body depth at dorsal-fin origin. Dorsal profile of body convex from snout

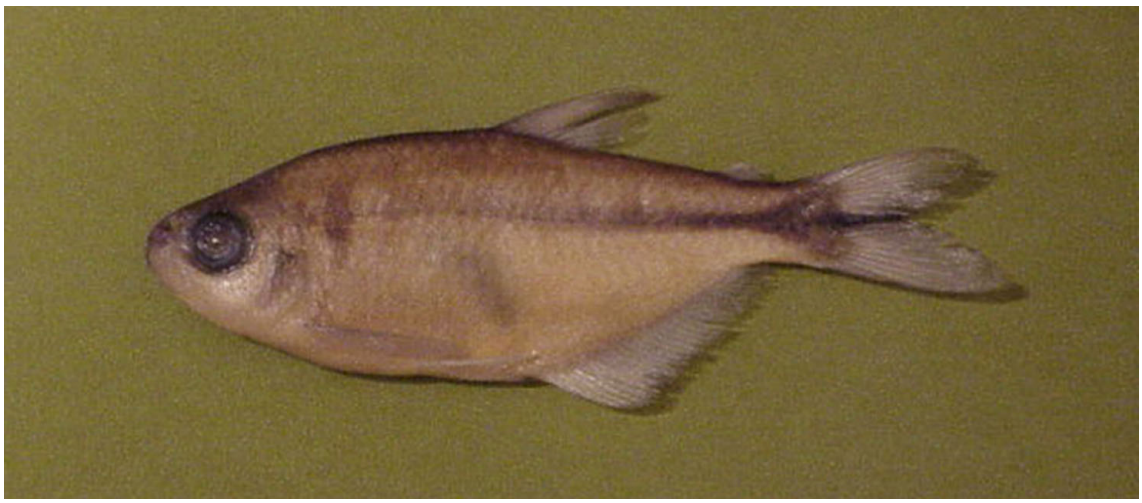

Figure 1. *Astyanax tupi* new species, holotype, MACN-Ict 8646, male, 70.1 mm SL, Argentina, province of Misiones, arroyo Cuñapirú, affluent of río Paraná.

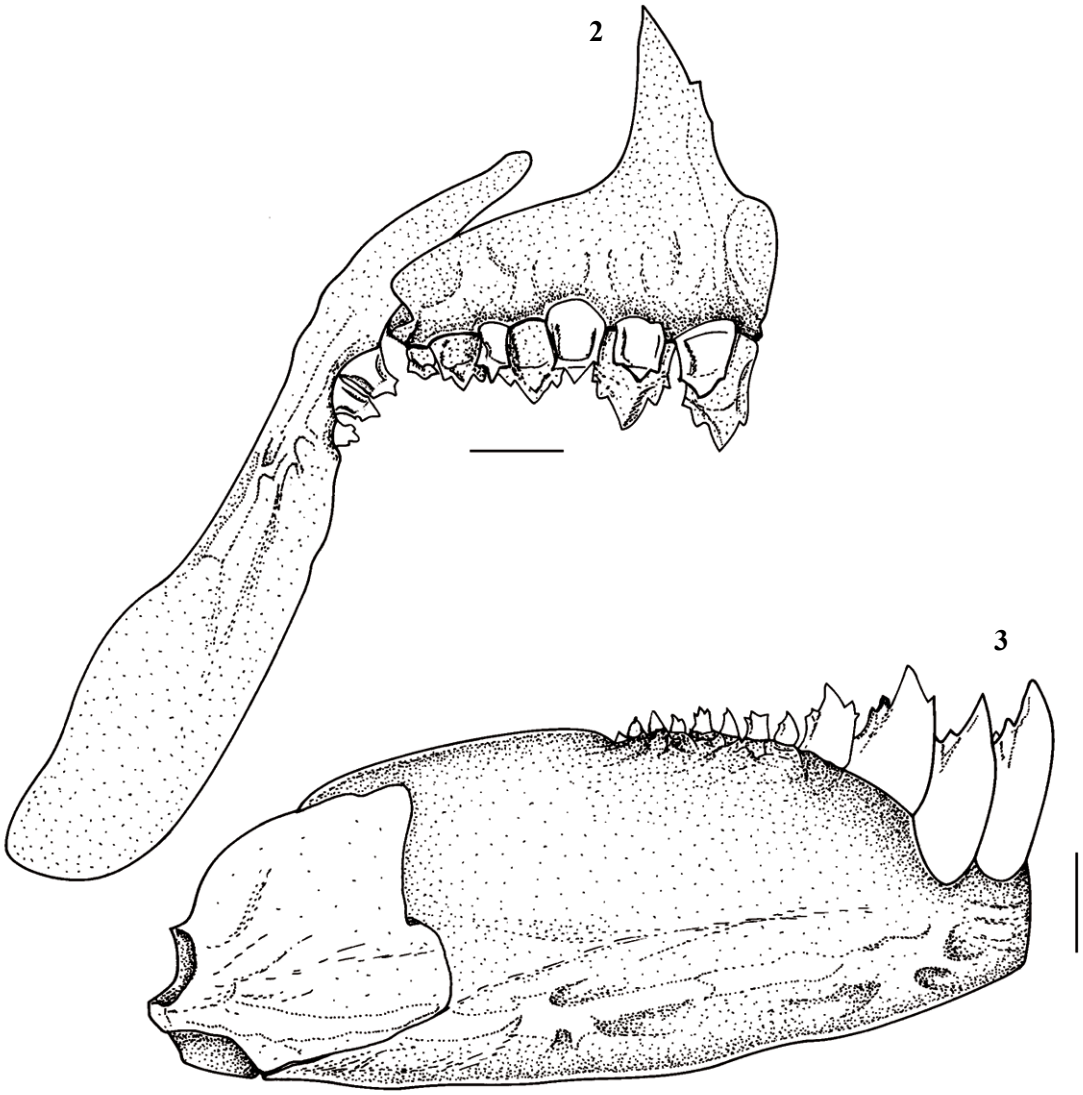

Figure 2. *Astyanax tupi*. n. sp., external view of premaxilla and maxilla. Scale= 1 mm. **Figure 3**. *Astyanax tupi* n. sp., external view of dentary. Scale= 1 mm.

tip to dorsal-fin origin, sligthly depressed on supraoccipital; slanted ventrally from dorsal-fin origin to caudal peduncle. Dorsal profile of caudal peduncle slightly concave or straight, ventral profile slightly convex or straight. Ventral profile of body slightly curved from tip of snout to pelvic-fin origin, almost straight between this point and anal fin origin, and slanted dorsally to caudal peduncle.

Dorsal-fin origin equidistant from snout tip and base of caudal-fin rays. Anal-fin origin at level of a vertical through fifth or sixth branched dorsal-fin rays. Tip of pectoral fin always surpassing one third or one fourth of pelvic-fin length, even in small specimens. Tip of pelvic fin surpassing anal-fin origin, reaching a vertical through first or second branched anal-fin rays in males; in small specimens and most females, pelvic fin reaching anal-fin origin.

Dorsal fin iii,9 rays, first unbranched ray only visible in cleared and stained specimens; posterior margin of dorsal fin straight; last unbranched and first branched dorsal-fin ray longest.

Anal fin iv-vi,24-27 rays (2 spms.= 24; 3 spms.= 25; 6 spms.= 26; 4 spms. including holotype= 27). Posterior margin straight in males; in females, last unbranched and first four to six branched rays forming a small lobe. Anal fin of males with broad hooks, placed on last unbranched and up to sixteen branched anal fin rays. One pair of hook on each segment, directed inward and slightly

Table 1. Morphometrics of the holotype (MACN-Ict 8646) and 14 paratypes of *Astyanax tupi* n. sp. Standard length expressed in mm; SD, standard deviation. Means include the holotype.

	Holotype	Range	Mean	SD
Standard length	70.1	60.8-73.2		
Percentages of SL				
Predorsal distance	57.2	52.9-58.7	55.6	1.3568
Preanal distance	62.6	59.0-66.8	62.4	1.8368
Prepelvic distance	46.2	44.0-49.6	46.0	1.3462
Body depth	39.3	36.9-42.6	39.6	1.4714
Dorsal fin base	14.5	12.8- 15.7	13.7	0.7431
Anal fin base	36.6	31.6-38.9	36.0	1.9993
Pectoral fin length	25.6	23.2-27.5	25.4	0.9862
Pelvic fin length	20.6	17.9-21.9	19.9	1.1082
Distance between pectoral and pelvic fin origins	17.9	16.8-21.8	18.7	1.0659
Distance between pelvic and anal fin origins	17.8	16.1-20.9	18.4	1.1444
Head length	28.8	25.9-29.5	28.7	0.7366
Peduncle depth	10.9	9.7-12.8	11.7	0.7199
Peduncle length	11.4	9.8-12.4	11.2	0.8293
Percentages of peduncle L				
Peduncle depth	96.2	94.4-121.6	104.7	8.8801
Percentages of HL				
Eye	39.6	35.2-45.2	39.8	2.4148
Interorbital distance	31.1	30.6-33.9	32.0	0.8462
Postorbital length	42.5	41.6-48.5	44.6	1.8656
Snout length	24.7	21.6-27.7	24.1	1.4754
Maxillary (M) length	28.2	24.8-31.7	28.5	1.5187
M+premaxillary length	42.5	39.3-47.3	42.1	1.9047

Table 2. Percentages of SL significantly different between *Astyanax tupi* n. sp., *Astyanax* sp. A, and *Astyanax* sp. C after a Mann-Whitney test, significance p<0,001. Shadowed values correspond to those significantly different of *A. tupi.* n. sp.

Percents of SL	*A. tupi*	*Astyanax* sp. A	*Astyanax* sp. C
Head length	25.9 - 29.5 (28.7)	25.8 - 28.5 (27.0)	27.2 - 30.3 (28.6)
Eye diameter	10.2 - 11.9 (11.1)	9.9 - 11.3 (10.6)	12.1 - 14.4 (13.0)
Interorbital length	8.5 - 10.0 (8.9)	7.3 - 8.9 (7.9)	7.7 - 9.0 (8.5)
Snout length	6.0 - 7.3 (6.7)	6.1 - 7.2 (6.4)	4.9 - 6.9 (5.6)
Maxillary length	7.3 - 8.9 (7.9)	8.2 - 9.7 (9.2)	6.9 - 9.0 (8.4)
Predorsal distance	52.9 - 58.7 (55.6)	51.7 - 55.6 (54.0)	51.7 - 55.9 (54.1)
Body depth	36.9 - 42.6 (39.6)	34.9 - 39.1 (37.4)	34.9 - 39.9 (35.6)
Peduncle depth	9.7 - 12.8 (11.7)	10.0 - 11.4 (10.7)	10.0 - 12.4 (11.4)
Pectoral to pelvic-fin origins	16.8 - 21.8 (18.7)	18.2 - 21.8 (20.0)	17.5 - 21.4 (20.1)

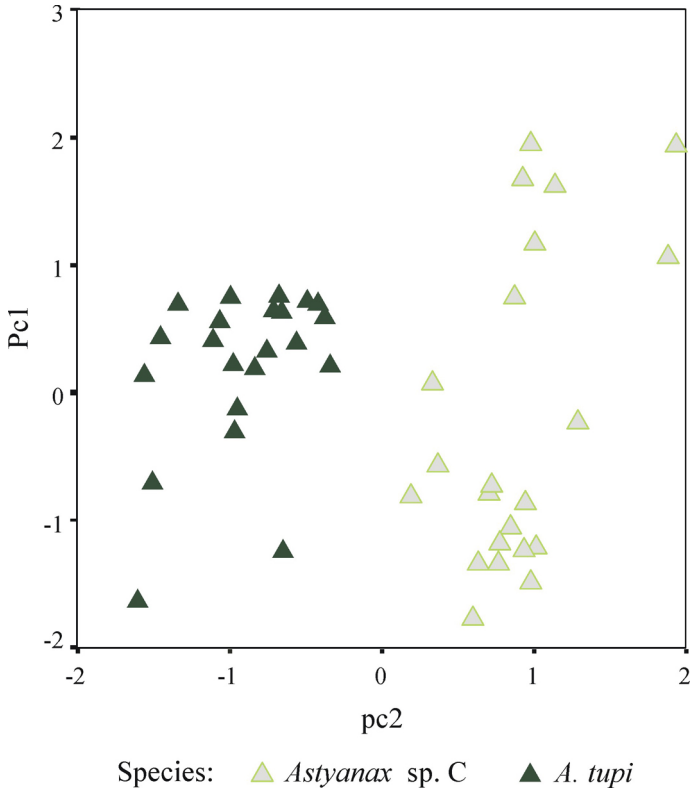

Figure 4. PCA showing *Astyanax* sp. C and *A. tupi*. The first component only indicates differences in size, while the second one discriminates between these species.

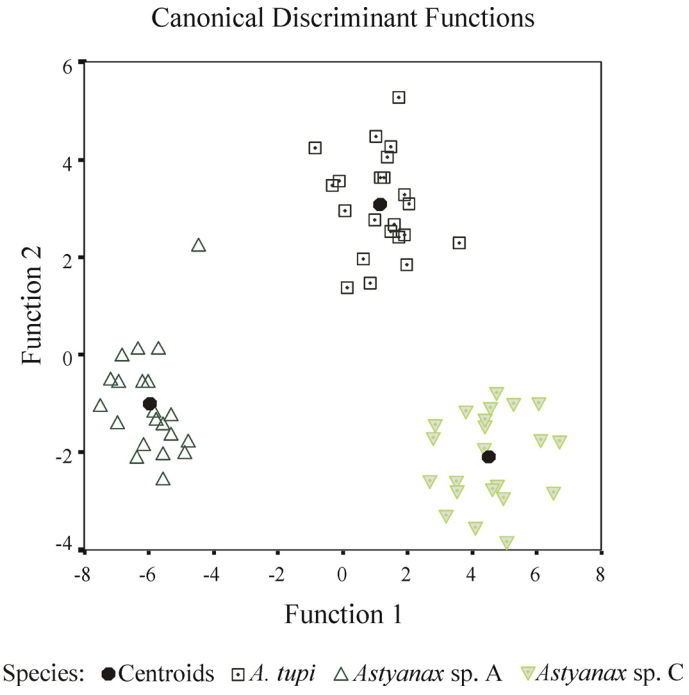

Figure 5. Canonical discriminant functions between *Astyanax* sp. A, *Astyanax* sp. C and *A. tupi* n. sp. The centroids represent the mean value s on the two axes among individuals of each species.

curved dorsally, in both ray branches although especially on posterior branch.

Caudal fin bearing 1 unbranched and 9 branched principal rays in upper lobe; 8 branched and 1 unbranched principal rays in lower lobe. Lower caudal lobe longer than upper one.

Pectoral-fin i,12-14 rays (7 specimens including holotype= 12; 5 spms.= 13; 3 spms.= 14); posterior margin slightly curved.

Pelvic fin i,7 rays; very small hooks developed on posterior branch of rays, occasionally on first unbranched ray also. Usually, one pair of hooks on each segment, most of them slightly curved inward.

Head length moderate, mouth terminal, horizontal; snout short. Lower jaw scarcely longer. Maxilla long, narrow, reaching near middle of eye. Eye large, interorbital broad, triangular supraoccipital process sorrounded by four scales. Third infraorbital almost contacting preopercular canal of lateral line system.

Premaxilla with narrow ascending process, and relatively long lateral process, bearing two series of teeth. Outer series with 3-5 tricuspidate teeth; when 4 or 5 teeth present, third tooth out of line in most specimens. Inner series of premaxillary teeth consisting of 5 teeth. Symphysial tooth narrower and deeper, with 3 or 4 cusps. Second, third, and fourth teeth with 5 cusps; sometimes fourth tooth with 4. Fifth tooth smaller, with 3 to 5 cusps, out of line of remaining teeth. In all teeth, central cusp larger than remainig ones. Two or three maxillary teeth with 3 to 5 cusps (Fig. 2). Dentary bearing 3 large pentacuspid teeth anteriorly, 1 median tooth tetracuspidate or pentacuspidate, followed by 7-8 (usually 8) teeth very small tricuspidate to conic (Fig. 3).

Scales cycloid, posterior margin straight. Lateral line with 35-38 perforated scales (1 spm.= 35; 3 spms.= 36; 10 spms. including holotype= 37; 1 spm.= 38). Six or 7 scales between dorsal-fin origin and lateral line; 5 scales between lateral line and ventral-fin origin. Thirteen or fourteen scales around caudal peduncle. Eleven to fifthteen scales forming an irregular row between supraoccipital process and dorsal-fin origin. Twelve to fifthteen rectangular scales placed on anal- fin base, covering all unbranched and fourteen or fifthteen branched anal-fin rays. Scales placed on basal fifth of caudal lobes. A narrow oval, long, axillary scale present dorsal to pelvic-fin insertion.

In three cleared and stained specimens first arch bearing 24-25 gill-rakers: 2 on hypobranchial, 7-8 on ceratobranchial, 1 on cartilage, and 13-14 on epibranchial. Total number of vertebrae 35; dorsal-fin pterygiophores between neural spines of vertebra 10-11 and 18-19; anal-fin pterygiophores between hemal spines of vertebrae 15-16 and 28-29. Five or 6 supraneurals. Eleven pairs of ribs. Caudal fin with 7-9 dorsal procurrent rays and 8-9 ventral procurrent rays.

Coloration of alcohol preserved specimens

Background pale yellow, darker above lateral stripe on flanks and dorsal surface of head. A humeral spot well developed, dorsoventrally expanded; a second lateral spot faint, just anterior to the vertical through pelvic-fin origin. A third rounded small spot, between posterodorsal opercular margin and first humeral spot; first spot indicating origin of lateral stripe. Dark lateral stripe very narrow near head, increasing its width beneath dorsal fin, finishing in a triangular spot on caudal peduncle. Lateral stripe with different intensity in coloration. A light area bounding posteriorly caudal spot. A narrow black band extending on middle caudal-fin rays to their tips. Most specimens with dark chromatophores on inner opercular surface forming a spot.

Dark chromatophores on distal margin of anal fin, forming a band; first unbranched dorsal-fin ray with black chromatophores on anterior face; scarce chromatophores, especially on membranes, increasing in numer on distal area of dorsal fin; tips of caudal-fin rays with dark chomatophores; pectoral fin with scarce chromatophores on proximal half; pelvic fins hyaline.

Morphometric comparisons

Most of the measurements of *A. tupi* are shared with *Astyanax* sp. A and *Astyanax* sp. C. The differences are presented in table 2.

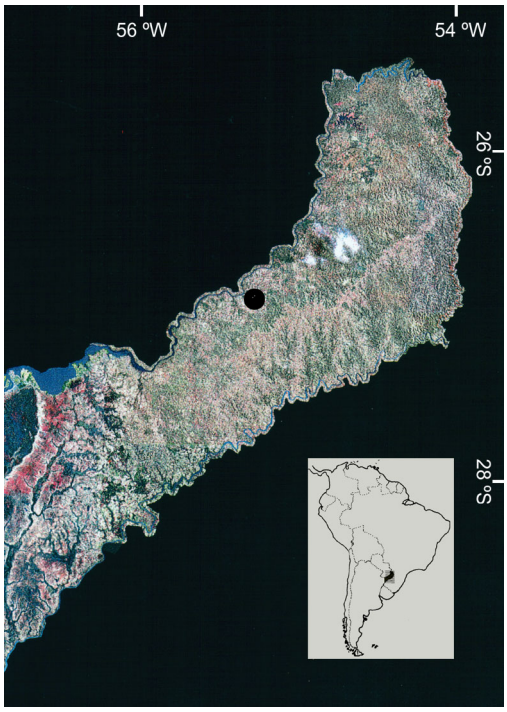

Figure 6. Map showing collecting localities of *Astyanax tupi* n. sp. (modified from a photograph of Instituto Geográfico Militar).

Table 3. Eigenvectors on first two principal components. Highest and lowest values on PC2 are highlighted in dark green and light green respectively. The variables with highest eigenvectors in PC2 are particularly high for *Astyanax* sp. C and variables with negative values are relatively high for *A. tupi* n. sp. Highest and lowest values are hightlighted in dark green and light green respectively.

Astyanax tupi vs. *Astyanax* sp. C	Eigenvectors	
	pc1	pc2
Standard length	0,989	0,050
Head length	0,972	0,119
Eye diameter	0,634	0,702
Interorbital length	0,939	-0,207
Postorbital length	0,668	-0,233
Snout length	0,870	-0,285
Maxillary length	0,874	0,316
Predorsal distance	0,982	-0,054
Preanal distance	0,956	0,154
Prepelvic distance	0,862	0,157
Dorsal-fin base	0,873	0,105
Anal-fin base	0,932	0,033
Body depth	0,952	-0,197
Peduncle depth	0,905	-0,070
Peduncle length	0,826	-0,297
Pelvic-fin length	0,815	-0,285
Pectoral-fin length	0,891	-0,056
Pectoral to pelvic-fin origins	0,839	0,323
Pelvic to anal-fin origins	0,924	-0,143

Figure 7. The stream Cuñapirú, near the place where *Astyanax tupi* n. sp. was collected.

The PCA shows differences between *A*. *tupi* and *Astyanax* sp. C (Fig. 4); the differentiation of both species is evident on component 2 in which *Astyanax* sp. C has positive values and *A*. *tupi* has negative ones. The eigenvalue of each individual on each component is given by the addition of products between used measurements and the eigenvectors of corresponding variables. Therefore, the specimens of *A*. *tupi* have high values for variables with negative eigenvectors, and low values for those variables with positive eigenvectors. In contrast, positive values are found in *Astyanax* sp**.** C. Eigenvectors showing relative heights of variables on each principal component are expressed in table 3. The PCA does not show notable differences between *A*. *tupi* and *Astyanax* sp. A.

Discriminant analyses were independently performed between *A*. *tupi*- *Astyanax* sp**.** A, and *A*. *tupi*- *Astyanax* sp**.** C to find the best ratio between variables to separate species. The ratio eye/SL is useful between *A*. *tupi* and *Astyanax* sp. C (10.2-11.9 vs. 12.2-14.4 % SL). The ratio maxillary length/interorbital width clearly separates *A*. *tupi* from *Astyanax* sp**.** A (100.0-136.6 vs. 81.2-90.7 % interorbital width). Standardized coefficient of canonical discriminant functions of those analyses are presented in tables 4 and 5.

Table 4. Standardized canonical discriminant function coefficients obtained with DA between *A. tupi* n. sp. and *Astyanax* sp. C. Variables with positive values are relatively high for *Astyanax* sp. C and those with negative values are relatively high for *A. tupi*. Highest and lower values are highlighted in dark green and light green respectively.

Standardized Canonical Discriminant Function Coefficients	
	Function 1
Standard length	-2,519
Eye diameter	2,046
Snout length	-1,066
Maxillary length	0,948
Body depth	-1,155
Pectoral to pelvic-fin origins	1,981

Table 5. Standardized canonical discriminant function coefficients obtained with DA between *A. tupi* and *Astyanax* sp. A. Variables with positive values are relatively high for *Astyanax* sp. A and those ones with negative values are relatively high for *A. tupi* n. sp. Highest and lowest values are highlighted in dark green and light green respectively.

Standardized Canonical Discriminant Function Coefficients	
	Function 1
Eye diameter	0,949
Interorbital length	0,849
Maxillary length	-0,963

Table 6. Standardized canonical discriminant function coefficients including *Astyanax* sp. A, *Astyanax* sp. C, and *A. tupi* n. sp. Highest and lowest values are highlighted in dark green and light green respectively.

Standardized Canonical Discriminant Function Coefficients		
	Function1	Function2
Eye diameter	2,058	-0,921
Interorbital length	1,144	0,680
Maxillary length	-0,930	-1,335
Predorsal distance	-1,876	2,393
Preventral distance	0,800	-0,820
Body depth	-0,582	1,176
Pectoral to pelvic-fin origins	0,064	-1,163

Functions at Group Centroids		
Species	Function1	Function2
Astyanax sp. C	4,517	-2,092
Astyanax sp. A	-5,959	-1,028
Astyanax tupi	1,171	3,074

A discriminant analysis including the three species was performed (Fig. 5) and coefficients of discriminant functions obtained are expressed in table 6.

Distribution

The new species is known from two different streams of the Paraná basin, the arroyos Cuñapirú Chico and Cuñapirú, in the province of Misiones, Argentina (Fig. 6, 7).

Etymology

The specific epithet *tupí* honors aborigines that lived in northern Argentina.

Discussion

A relatively high diversity of *Astyanax* were recorded in southern Brasil and the northeast of Argentina. Among those species of *Astyanax*, there is a small group formed by *A. leonidas*, *A. ojiara, A. troya*, and *Astyanax* sp. B which bear hooks on anal, pectoral, pelvic, and caudal fins, a fact that clearly discriminate them from *A. tupi*.

The high total number of anal fin rays of *A. pelegrini* (41-48 per. obs.), *A. correntinus* (45), and *A. erythropterus* (45) (two last values taken from the original description) distinguish them from *A. tupi* (28-33). Also, the number of anal rays of *A. tupi* separates it from other species of the **r**ío de la Plata basin and southeastern Brazil, e.g., *A. gymnogenys* (21-22), *A. cremnobates* (18-22), *A. brachypterygium* (16-20), *A. scabripinnis paranae* (17-23), *A. ribeirae* (23-27), and *A. paris* (20-22) (Eigenmann, 1921, 1927; Garutti & Britski, 2000; Bertaco & Malabarba, 2001; Azpelicueta *et al.*, 2002b).

The numerous lateral stripes developed on the flanks of *A. lineatus* differentiate it from *A. tupi* which bears a single lateral stripe. The three spots on the flanks of *A. tupi* distinguish it from *A. laticeps* which has a single oval humeral spot.

The presence of 2-3 maxillary teeth in *A. tupi* separates it from *A. eigenmanniorum*, *A. cf. fasciatus*, and *A. marionae* with only one maxillary tooth, and from *A. asuncionensis, A. cordovae*, and *A. abramis* without maxillary teeth.

The orbital diameter of *A. tupi* (35.2-45.2 % HL) is similar to those of *A. ita* (38.6-42.0 % HL), *Astyanax* sp. C (41.1-45.5 % HL), and *Astyanax* sp. A (35.9-42.1 % HL). Nonetheless, the heptacuspidate maxillary tooth and the number of branched anal fin rays of *A. ita* (20-24) clearly differentiate it from the remaining species.

Astyanax tupi, *Astyanax* sp. A, and *Astyanax* sp. C share long pectorals, long anal fin with a similar number of branched rays, anal fin origin placed below dorsal fin, similar number of perforated scales on lateral line, and similar shape of teeth. The discriminant analysis found the best ratio- expressed as percentages- to separate them: eye/SL is useful between *A. tupi* and *Astyanax* sp. C (10.2-11.9 vs. 12.2-14.4 % SL), and maxillary length/interorbital width clearly separates *A. tupi* from *Astyanax* sp. A (100.0-136.6 vs. 81.2-90.7 % interorbital width).

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