

***Papiliolebias hatinne*, a new annual fish species
(Cyprinodontiformes: Rivulidae) from Salta, Argentina**

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***Papiliolebias hatinne*, a new annual-fish species (Cyprinodontiformes: Rivulidae) from Salta, Argentina.** - *Papiliolebias hatinne* sp. n. is described from a temporary pond, 5 km north of Embarcación, in the río Bermejo basin, Province of Salta, Argentina. *Papiliolebias hatinne* sp. n. is distinguished from the only congener, *P. bitteri*, from the río Paraguay basin, by a combination of characters: 26-28 vertebrae, with 8-10 being pre-caudal; second proximal radial of anal fin located between vertebrae 9 and 10 or 10 and 11; 1-2 vomerine teeth; an elongation of jaw suspensorium; first epibranchial somewhat triangular; 7-8 pelvic-fin rays; 12-13 pectoral-fin rays; 20-23 caudal-fin rays; males with anal fin turquoise blue, dorsal and caudal fins bluish, pelvic fin blue, and humeral spot metallic bluish green; females without dark dots on flanks. The chromosome formula is $2n=28$, the $FN=36$, integrated by two pairs of metacentric, two pairs of submetacentric and ten pairs of subtelocentric chromosomes.

Keywords: new *Papiliolebias* - Bermejo basin - karyogram of *Papiliolebias hatinne*.

INTRODUCTION

The tribe Plesiolebiasini Costa (1998a; 2007) includes members of the genera *Papiliolebias*, *Pituna*, *Plesiolebias*, *Maratecoara*, and *Stenolebias*. Among those genera, only the *Papiliolebias* Costa, 1998 is endemic to the Chaco subregion. The genus *Papiliolebias* was monotypic including only *Papiliolebias bitteri* (Costa, 1989). This species is common in environments of the Paraguayan Chaco, included in the lower Paraguay river basin, together with further rivulid species such as *Austrolebias chacoensis* (Amato, 1986), *A. vandenberghi* (Huber, 1995), and *Trigonectes balzanii* (Perugia, 1891) among others.

One of the longer southernmost affluent into Paraguay river in Argentinean Chaco subregion is the río Bermejo. Some collections close to the río Bermejo yielded specimens of a new rivulid species which has been included in the genus *Papiliolebias*.

The objective of the present paper is the description of *Papiliolebias hatinne* sp. n. collected in one temporary pond close to the río Bermejo, in the province of Salta, Argentina.

MATERIAL AND METHODS

The measurements were taken with a dial caliper, following Costa (1988). Proportions are expressed as percentages of different lengths (SL and HL), or otherwise as specified. Counts indicated the total number of fin rays in all fins. Specimens were cleared and counterstained following Taylor & Van Dyke (1985). Bivariate and multivariate analyses were performed with Statistica 5.5 and SPSS, respectively. Measurements of *Papiliolebias bitteri* were taken from Costa (1998a). Institutional acronyms follow Fricke & Eschmeyer (2008).

COMPARATIVE MATERIAL EXAMINED: *Papiliolebias bitteri* (Costa, 1989): ANSP 170422, 2 males, 2 females, Paraguay (not measured); MCP 12794, paratypes, 1 male, 1 female (photographs), Paraguay.

CYTOGENETIC METHODS: Eleven individuals (6 males, 5 females) belonging to natural populations were analyzed. Metaphases were studied following the protocols of Kligerman & Bloom (1977) with modifications proposed by Bertollo *et al.* (1978). The better metaphase preparations from spleen, kidney, and gill epithelium were selected under a microscope Nikon Microphot-Fx with and immersion objective. Photographs were taken with Kodalith (25 asas). Chromosome nomenclature follows Levan *et al.* (1964) and Denton (1973) for fishes. Based on arm relation (AR), chromosomes were classified as metacentric (M), submetacentric (SM), subtelocentric (ST), and acrocentric (A). The diploid number (2n) and the number of chromosome arms (FN) were also established.

RESULTS

Papiliolebias hatinne sp. n.

Figs 1, 3-5, Tables 1-3

Holotype: FML 4000, male, 32.4 mm SL, Argentina, Province of Salta, río Bermejo basin, temporary pond at the side of Ruta Nacional 34, 5 km north of Embarcación, close to 23° 15'S - 64° 07'W, coll. C. Butí, F. Cancino, and E. Lavilla, May 1999 (Fig. 1).

Paratypes: The thirty four specimens studied came from Argentina, Province of Salta, and were collected in the same locality of the holotype by C. Butí, F. Cancino, and E. Lavilla. FML 4501, 5 ♂, 26.3-32.7 mm SL, 5 ♀, 28.1-30.4 mm SL, collected with the holotype. FML 4502, 3 ex. C&S, 2 ♂, 29.15-30.5 mm SL, 1 ♀, 27.8 mm SL, March 1999. FML 2786, 4 ♂, 22.8-25.8 mm SL. FML 2963, 1 ♂, 35.3 mm SL, 10 ♀, 26.6-35.6 mm SL. MHNG 2704.014, 1 ♂, 24.8 mm SL, 5 ♀, 24.4-26.1 mm SL, May 1999. Genetic samples were obtained from FML 2963 specimens and stored at the Sección Genética Evolutiva, Facultad de Ciencias, Universidad de la República, Uruguay, catalogue number R35-44.

DIAGNOSIS: *Papiliolebias hatinne* sp. n. is distinguished from *P. bitteri* –the sole species of the genus– by a combination of characters: 26-28 vertebrae with 8-10 of them precaudal; second proximal radial of anal fin located between vertebrae 9 and 10 or 10 and 11; 1-2 vomerine teeth; an elongation of jaw suspensorium; first epibranchial somewhat triangular; 7-8 pelvic-fin rays; 12-13 pectoral-fin rays; 20-23 caudal-fin

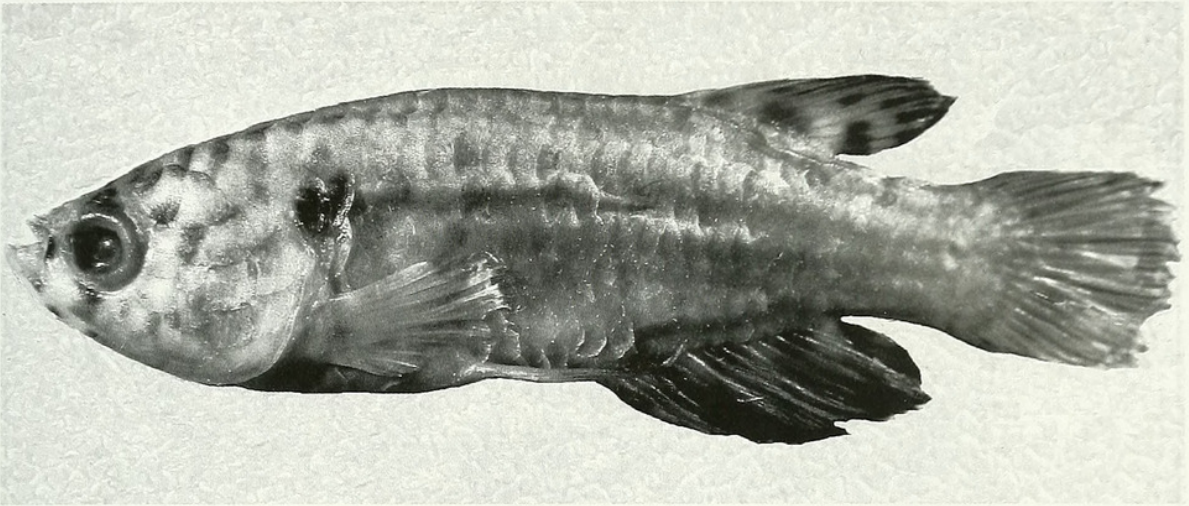


FIG. 1. *Papiliolebias hatinne* sp. n., holotype, male, FML 4000.

rays; males with anal fin turquoise blue, dorsal and caudal fins bluish, pelvic fin blue, and humeral spot metallic bluish green; females without dark dots on their flanks.

DESCRIPTION: Morphometric and meristic data of the holotype and 10 paratypes are presented in Tables 1-3. Females are larger than males in studied specimens (47.6 mm, 35.3 mm maximum of SL respectively). Dorsal profile of body convex from tip of snout to dorsal-fin origin; gently concave caudal peduncle in males, almost straight in females. Anteroventral profile of head gently rounded. Ventral profile of body very gently convex, caudal peduncle ventrally convex in males and almost straight in females. Some males with concavity on frontal and supraoccipital areas; females always straight.

Dorsal-fin rays 11-12 (iii,8,i or iii,8, one specimen with iii,7) in both sexes. Tip of dorsal fin rounded in both sexes without filamentous rays; dorsal fin origin in a vertical through 6th to 9th anal-fin rays. Caudal fin with 20-23 rays (iv,12-14,iv-vi); caudal-fin margin rounded, central rays longer than upper and lowermost rays. Anal-fin rays with 17-19 (iii,14-16 or iii,15,i) in males; in females, 16-18 rays (ii,14-16). Anal fin rounded, anterior and posterior portions of fin with similar length in both sexes. Pectoral fin with 12-13 rays (i,10,i or i,11,i); distal margin of pectoral fin rounded, with three middle rays notably longer, falling in a vertical through pelvic-fin base and urogenital papillae in males; in females, tip of pectoral fin reaching base of pelvic fin. Pelvic fin with 7-8 rays (i,5,i or i,6 or i,6,i), with 3rd ray extended as filament, finishing between 7th and 11th anal-fin rays in males; in females, longest ray reaching 2nd to 4th anal fin ray.

Scale pattern variable; most specimens with E-scales overlapping; frontal squamation circularly ordered; A-scale without free margins, situated between eyes or completely posterior to them. Sometimes, A-scale at lower level than remaining scales. Both sides of body with one predorsal longitudinal obtuse keel; series of scales forming keel with soft central region, especially developed in live males. Longitudinal series with 26-28 scales; transverse series with 11 scales; fourteen scales around caudal peduncle. Supraorbital neuromasts 7-8+4.

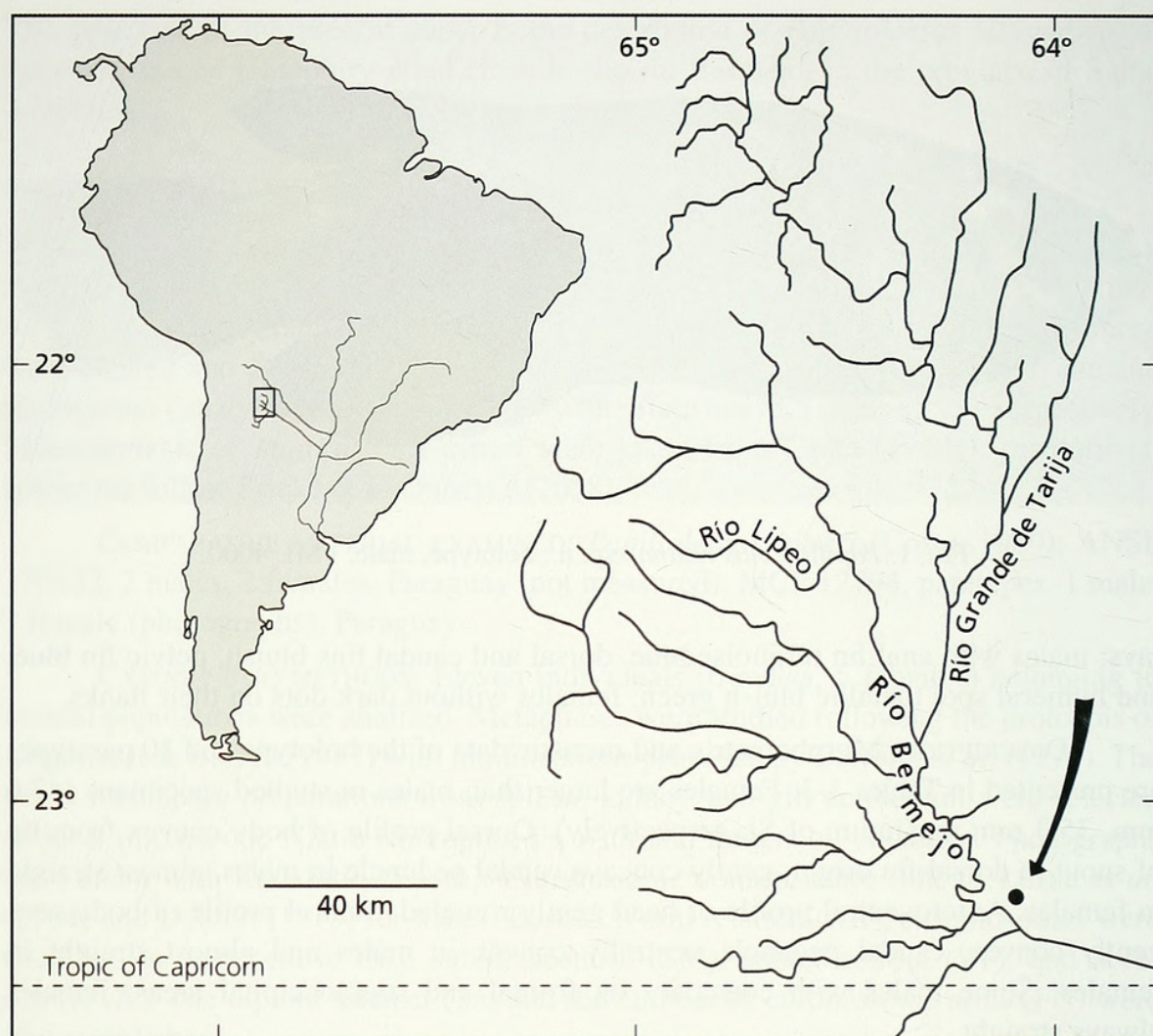


FIG. 2

Map indicating the type locality of *Papiliolebias hatinne* sp. n., close to 23° 15' S - 64° 07' W, río Bermejo basin, Province of Salta, Argentina.

COLOUR IN LIFE: Male: Background iridescent gold excluded dorsal and peduncular areas iridescent bluish. Flanks with 5 to 8 oblique irregular black stripes, some of them forming a V with vertex oriented ventrally. Dorsum and side of head golden beige, with dark spots; iris orange; dark wide suborbital bar, reaching isthmus; opercular region golden. Humeral spot metallic bluish green on 2-4 scales. Dorsal and caudal fins bluish and iridescent; sometimes both fins with dark gray margins; three large black spots on dorsal-fin base; small dots scattered over complete surface of fin. Anal fin turquoise blue, distal margin white; 5-6 dark large basal spots alternating with white spots. Pectoral fins hyaline, pale yellowish. Pelvic fins blue. Chromatophores concentrated on posterior scale margin, forming a reticulate pattern.

Female: Flanks of body and head pale beige, iridescent, without dark spots. Unpaired fins hyaline; some specimens with those fins grayish or yellowish. Paired fins hyaline; in some specimens, pelvic fins yellowish. Iris pale orange; suborbital bar dark, slender and short; opercular and humeral areas golden; a translucent portion on gill

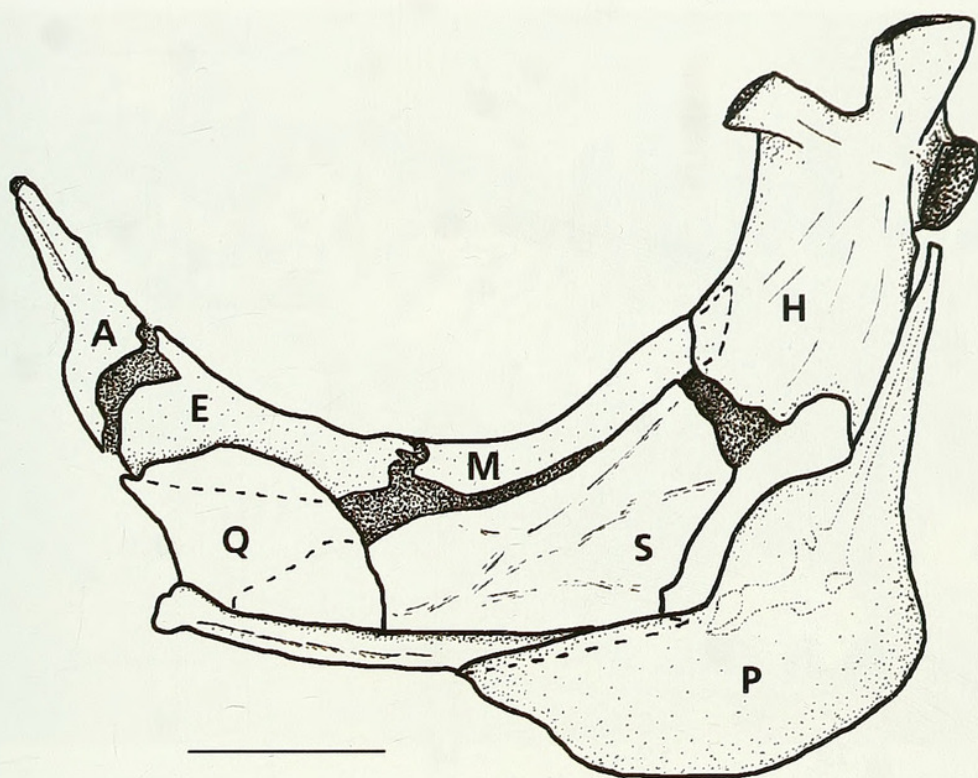


FIG. 3

Suspensorium of *Papiliolebias hatinne* sp. n., FML 4002, 27.8 mm SL. A= autopalatine; E= ectopterygoid; H= hyomandibula; M= metapterygoid; P= preopercle; Q= quadrate; S= symplectic.

rakers. Chromatophores concentrated on central scale portion, forming a cobblestone-like surface.

ETIMOLOGY: The specific epithet *hatinne* means nice in the indigenous Chorote language. The word is used as an adjective in apposition.

DISTRIBUTION: *Papiliolebias hatinne* sp. n. is known to be present in only the type locality, one temporary pond close to río Bermejo basin, at the side of Ruta Nacional 34, 5 km north of Embarcación (close to 23° 15'S - 64° 07'W), Salta, Argentina (Fig. 2).

HABITAT: The pond was 20 m long, 6 m wide, and 1.2 to 1.5 m deep with clear water; its surface was almost completely covered by duckweed (*Lemna* sp.), floating fern (*Salvinia* sp.), water lettuce (*Pistia*), and water hyacinth (*Eichornia* sp.). The margins of the pond had terrestrial macrophytes. The composition of vegetation on the surface of ponds along different periods of the year in the Chaco form the "pastizal de los madrejones" (pasture of the swamps; Morello & Saravia Toledo, 1959) at the beginning of the rainy season to the "camalotal" (place with the aquatic *Eichornia*; Burkart, 1957) at the end of that period.

The Chaco area has a subtropical climate with warm summers and temperate winters; annual mean temperature varies between 14 to 26 °C; the annual rainfall reaches 500-1,000 mm, but with a marked rainy season from October to March followed by a relative dry period. The water of many temporary ponds completely evaporates during dry season (Cabrera, 1971; Morello & Adamoli, 1974).

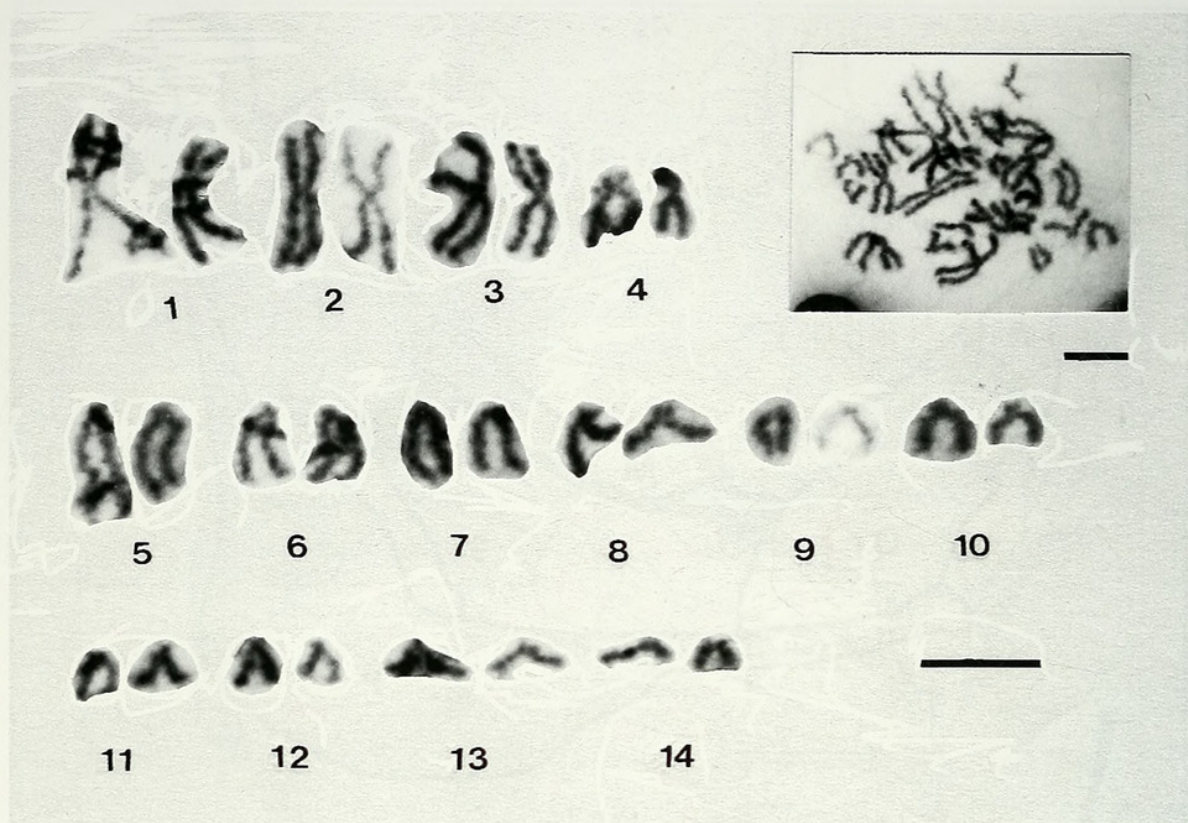


FIG. 4

Conventional karyograms of somatic cells of *Papiliolebias hatinne* sp. n., $2n=28$, $NF=36$, the chromosome formula was $M-SM=8$, $ST=20$. Bar= 10 μ m.

OSTEOLOGICAL CHARACTERS: Three cleared and stained specimens of *P. hatinne* sp. n. were examined. They have 26-28 vertebrae, 8-10 being precaudal and 16-18 caudal; 9-10 pairs of ribs; dorsal fin with second proximal radial placed between neural spines of vertebrae 13 and 14 or 14 and 15; anal fin with second proximal radial located between vertebrae 9 and 10 or 10 and 11.

First branchial arch with 8 or 9 gill-rakers distributed as follows, 1 on epibranchial and 7-8 on ceratobranchial. Epibranchial 1 somewhat triangular; epibranchials 2 and 3 slender and short. Interarcual cartilage present, very small.

Supraoccipital spine reachig first neural spine by ligaments; first neural spine broad or narrow. Ventral process of posttemporal very well developed but very short. One or two vomerine teeth.

Jaw suspensorium (Fig. 3) relatively long; symplectic long and somewhat rectangular; autopalatine posteriorly slender; quadrate with a long posterior process; deep vertical portion of preopercle with 2-3 pits of the sensory preopercular canal and 3-4 pits on horizontal ramus.

CYTOLOGICAL CHARACTERS: Somatic mitotic metaphase of *P. hatinne* sp. n. presented as $2n=28$ $FN=36$ (Fig. 4). Chromosome complement was composed of two pairs of metacentric, and two pairs of submetacentric chromosomes and ten pairs of subtelocentric chromosomes. Acrocentric chromosomes were not detected.



FIG. 5

Papiliolebias hatinne sp. n., paratype male in aquarium, FML 4501, 32.7 mm SL.

DISCUSSION

Costa (1989) described the genus *Plesiolebias* including one new species, *P. bitteri*, based on alive material exported from Paraguay to Germany for the aquarium enthusiasts. Subsequently, the author established the monotypic subgenus *Papiliolebias* (1998a) for this species, which taxonomical category was later raised to the generic level (1998b); the author listed a large number of synapomorphies that differentiate both genera. More recently, Costa (2007) redefined the tribe Plesiolebiasini and included in it the genera *Papiliolebias*, *Plesiolebias*, *Pituna*, *Stenolebias* and *Maratecoara*. The members of the tribe are distinguished from all other rivulids by unique features such as rostral cartilage with a concave anterior edge, a short ventral process of the angulo-articular, nonoverlapping of mesopterygoid and autopalatine, a bent first epibranchial, and a chorion surface covered by hooks.

Huber (1995) undertook a redescription of the species *P. bitteri* [sub *Cynolebias* (*Plesiolebias*) *bitteri*], listing seven localities for *P. bitteri* in the northwest of Paraguay and restricting the type locality to 64 km north of Mariscal Estigarribia, 21°03'S, 60°30'W, Nueva Asunción, Paraguay.

The genus *Papiliolebias* (Costa, 1998b) is diagnosed by four synapomorphies: 1- nine pelvic fin rays, 2- unpaired fins dark blue, 3- presence of a white stripe along the distal margin of the anal fin, and 4- a humeral metallic green spot. Both *Papiliolebias* species share the synapomorphies 3 and 4.

Nine pelvic fin rays are present exclusively in *P. bitteri* since *P. hatinne* has 7-8, a character considered plesiomorphic by Costa (1998a, 1998b). The unpaired fins dark blue only develop in *P. bitteri*, as these fins are simply bluish in *P. hatinne*.



FIG. 6

Papiliolebias bitteri, photograph by F. Bitter. An aquarium male specimen belonging to the original sample imported from Paraguay on which the species was based.

Thus the characters 1 and 2 may be considered as autapomorphies of *P. bitteri*. We have assigned the new species to *Papiliolebias* instead of creating a new genus, since we consider this choice more conservative.

The bivariate and multivariate analyses indicated differences sufficiently large as to enable a discrimination of *P. hatinne* from *P. bitteri*; the values obtained correspond to shorter predorsal length in both sexes (males 62.1-67.7 vs. 67.9-71.4, females 63.9-69.8 vs. 70.4-73.4), shorter prepelvic length in males only (44.9-46.8 vs. 47.4-51.5), and relatively longer head length in females only (27.4-28.9 vs. 26.6-27.8; Table 2).

Comparisons between *P. hatinne* and *P. bitteri* with regard to the number of caudal and pectoral fin rays and pigmentation pattern of scales, also exhibit significant differences between the two species. In both males and females of *P. hatinne*, the caudal fin has 20-23 rays (vs. 25-28 rays) and the pectoral fins have 12-13 (vs. 14-15; Table 3).

With respect to the pigmentation patterns, the anal fin of the males is turquoise blue in *P. hatinne* (Fig. 5) but dark blue in *P. bitteri* (Fig. 6), while the metallic humeral spot of the males is bluish green in *P. hatinne* and very dark blue in *P. bitteri* (Bitter, 1987, page 64). The minute dark-gray dots on the flanks of the *P. bitteri* females, cited by Costa (1989) are absent in the females of *P. hatinne*. The photograph of the original material from the paper in which *P. bitteri* was described (Bitter, 1987), however, does

TABLE 1. Morphometric data of holotype and 10 paratypes (5 ♀, 5 ♂) of *Papiliolebias hatinne* sp. n.

	Holotype	Males		Females	
		min	max	min	max
SL	32.35	26.3	32.7	28.10	30.35
Percents of SL					
Body depth	29.67	24.77	30.61	25.63	28.11
Caudal-peduncle depth	15.15	14.57	15.99	11.86	14.54
Predorsal length	67.23	62.12	67.68	63.92	69.80
Prepelvic length	45.59	44.88	46.86	48.22	51.30
Dorsal-fin base	18.24	17.95	19.58	15.00	17.46
Anal-fin base	27.36	26.60	29.66	22.34	26.02
Head length	29.21	28.54	31.94	27.43	28.90
Percents of HL					
Head depth	94.71	85.12	94.71	78.52	87.65
Head width	64.02	59.52	67.95	63.80	68.83
Eye diameter	32.8	28.11	35.26	33.33	39.61

TABLE 2. Morphometric data of *P. hatinne* sp. n. (5 ♀, 5 ♂) and *P. bitteri* (data taken from Costa 1998a). Discriminant values indicated with an asterisk.

	Males		Females	
	<i>P. hatinne</i>	<i>P. bitteri</i>	<i>P. hatinne</i>	<i>P. bitteri</i>
SL	26.3-32.7	33.0-34.4	28.1-30.3	27.6-30.4
Percents of SL				
Body depth	24.8-30.6	29.2-31.3	25.6-28.1	24.4-29.6
Caudal-peduncle depth	14.6-16.0	14.4-16.1	11.9-14.5	13.5-15.6
Predorsal length	62.1-67.7	67.9-71.4	63.9-69.8	70.4-73.4
Prepelvic length	44.9-46.9	47.4-51.5	48.2-51.3	49.3-51.7
Length of dorsal fin base	17.9-19.6	15.4-20.6	15.0-17.5	14.0-16.3
Length of anal fin base	26.6-29.7	26.9-32.5	22.3-26.0	23.9-27.3
Head length	28.5-32.0	27.3-29.5	27.4-28.9	26.6-27.8
Percents of HL				
Head depth	85.1-94.70	85.4-100.8	78.5-87.7	85.0-88.1
Head width	59.5-68.0	61.1-66.4	63.8-68.8	66.5-71.8
Eye diameter	28.1-35.2	31.9-36.5	33.3-39.6	35.2-37.5

TABLE 3. Meristic data of *P. hatinne* sp. n. (holotype, 5 ♀, 5 ♂) and *P. bitteri* (counts include values taken from Costa, 1989 and Huber, 1995). *The variation in number of longitudinal scale series for *P. bitteri* is 23-26 following Huber (1995).

	Males		Females	
	<i>P. hatinne</i>	<i>P. bitteri</i>	<i>P. hatinne</i>	<i>P. bitteri</i>
Pectoral-fin rays	12 - 13	14 - 15	12 - 13	14 - 15
Pelvic-fin rays	7 - 8	9	7 - 8	9
Dorsal-fin rays	10 - 12	10 - 12	10 - 12	11
Anal-fin rays	17 - 20	18	17 - 18	17 - 18
Caudal-fin rays	21 - 22	25 - 28	20 - 23	25 - 28
Longitudinal scales*	26 - 30	28 - 29	27 - 28	27 - 28
Transverse scales	11	9 - 11	11	9 - 11
Peduncular scales	14	13 - 15	14	13 - 15

not have dark-gray dots on the flanks; we did not examined fresh specimens of *P. bitteri*. Bitter (1987) commented that some specimens had parasites on the flanks, a condition that is, in fact, evident on examination of the flanks of MCP 12794.

Examination of the jaw suspensorium of *P. hattine* reveals an elongation relative to that of *P. bitteri* (See Fig. 4 present paper and Fig. 2a, in Costa 1998a), a more slender autopalatine, a relatively longer symplectic, and a quadrate with a longer posterior process than that of *P. bitteri*. Furthermore, *P. hattine* has 1-2 vomerine teeth, *P. bitteri* 1-4.

In *P. hattine* the first epibranchial has a wide posterior portion, being somewhat triangular as opposed to one that is completely wide in *P. bitteri*, whereas epibranchials 2 and 3 are slender in *P. hattine* but wide in *P. bitteri*.

Papiliolebias hattine has 8-10 precaudal vertebrae (vs. 12-13 in *P. bitteri*) and the second proximal radial of anal fin is located between pleural ribs of vertebrae 9-10 or 10-11 (vs. 10-13 in *P. bitteri*).

The present study constitutes the first report providing chromosome data of the genus *Papiliolebias*. Noteworthy is the observation that the diploid number of *P. hattine* is the lowest found among rivulid genera (Elder *et al.*, 1993; García *et al.*, 2001). The karyotype of *P. hattine* with the extra-large biarmed metacentric-submetacentric chromosomes is consistent with the previously proposed chromosome-evolution hypothesis (Scheel, 1972; García *et al.*, 2001).

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