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Early ontogeny of tetra *Markiana nigripinnis* (Characiformes: Characidae)

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The early development of *Markiana nigripinnis* is described by morphological characters, pigmentation, and morphometry. Larvae were obtained through semi-natural breeding, collected, fixed, and identified according to their development. Eighty individuals with standard lengths ranging from 3.1 to 24.3 mm were analyzed. Larvae are poorly developed at hatching, with a relatively large yolk sac and finfold. At the preflexion stage, the eyes are pigmented, the mouth and anus are functional, the yolk is completely absorbed, and the pectoral fin bud emerges. At flexion, the first rays of the caudal, anal, and dorsal fins become evident. The pelvic fin bud emerges only at the postflexion stage, in addition to the complete absorption of the finfold. Pigmentation is distributed throughout the body, with a greater concentration on the top of the head, around the mouth, and at the base of the caudal fin. The myomere total number ranged from 34 to 49 (16–23 preanal, and 18–27 postanal). Juveniles show morphological characteristics like adults. The fins ray number are pectoral: 11–13, pelvic: 5–7, dorsal: 8–11, caudal: 16–27, and anal 30–47. The morphometric relationships reveal variations in growth along the early ontogeny of the species.

Keywords: Fish, Ichthyoplankton, Juvenile, Lambari-do-campo, Larvae.

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O desenvolvimento inicial de *Markiana nigripinnis* foi descrito considerando os caracteres morfológicos, a pigmentação e a morfometria. Indivíduos foram obtidos por meio de reprodução seminatural, coletados, acondicionados, fixados e identificados conforme seu período e estágio de desenvolvimento. Foram analisados 80 indivíduos com comprimento padrão variando de 3,1 a 24,3 mm. As larvas são pouco desenvolvidas à eclosão, com saco vitelino relativamente grande e presença de membrana embrionária. Em pré-flexão, os olhos estão pigmentados, a boca e o ânus são funcionais, o vitelo é completamente absorvido e surge o botão da nadadeira peitoral. Em flexão, os primeiros raios das nadadeiras caudal, anal e dorsal tornam-se evidentes. O botão da nadadeira pélvica aparece somente em pós-flexão, além da completa absorção da nadadeira embrionária. A pigmentação se distribui pelo corpo todo, com maior concentração no topo da cabeça, ao redor da boca e na base da nadadeira caudal. O número total de miômeros variou de 34 a 49 (16–23 pré e 18–27 pós-anal). Os juvenis apresentaram características morfológicas semelhantes ao adulto. O número de raios das nadadeiras é peitoral: 11–13, pélvica: 5–7, dorsal: 8–11, caudal: 16–27 e anal 30–47. As relações morfométricas revelam variações no crescimento ao longo da ontogenia inicial da espécie.

Palavras-chave: Ictioplâncton, Juvenil, Lambari-do-campo, Larvas, Peixes.

INTRODUCTION

The Neotropical region is home to a high diversity of freshwater fish, with a rapid and ascending rate of description, going from approximately 5,000 valid species in 2003 (Reis *et al*., 2003), to 6,080 species in 2020 (Albert *et al*., 2020) and estimates that exceed 8,000 species (Ribeiro *et al*., 2011; Reis *et al*., 2016). The order Characiformes is the dominant group among the South American freshwater fish and is characterized by a body covered with scales, pelvic fins usually located well behind the insertion of the pectoral fins, soft fin rays, and, generally, the presence of an adipose fin (Britski *et al*., 2007; Nelson *et al*., 2016; Mirande, 2019). In this order, the family Characidae has 1,244 valid species, of which 221 have been described in the last decade, being among the ray-finned fish with the highest number of species (Mirande, 2019; Fricke *et al*., 2022). Species belonging to this family show a wide geographic distribution, from the southwestern United States to northern Patagonia, Argentina, in addition to being especially diverse in tropical South America, including small species ranging in size from 8 to 20 cm to large species over 1 meter (Marinho, 2017; Mirande, 2019; Ferreira *et al*., 2021).

The genus *Markiana* was proposed by Eigenmann (1903) for the previously described *Tetragonopterus nigripinnis* (Perugia, 1891), with only two known species: *Markiana geayi* (Pellegrin, 1909), restricted to the Orinoco River basin (Reis *et al*., 2003) and *Markiana nigripinnis* (Perugia, 1891). It has a disjunct geographic distribution and is considered *incertae sedis* (Lima *et al*., 2003); however, there are proposals that *Markiana* is closely related to some species, such as *Astyanax abramis* (Jenyns, 1842), *Astyanax asuncionensis* Géry, 1972 (synonym of *Astyanax lacustris* (Lütken, 1875) and *Astyanax*

lineatus (Perugia, 1891), and may also be considered a sister species to *Hyphessobrycon poecilioides* Eigenmann, 1913 (Baicere-Silva *et al*., 2011; Mirande, 2019). Research has been conducted on sperm ultrastructure (Baicere *et al*., 2011), molecular data (Oliveira *et al*., 2011; Thomaz *et al*., 2015), combined morphological and molecular data (Mirande, 2019) and cytogenetic analysis (Monteiro *et al*., 2022), suggesting the addition of *M. nigripinnis* among the Characidae in the subfamily Stervardiinae. Also known as tetra (lambari do campo in Portuguese), *M. nigripinnis* is found in the basins of the Paraná, Paraguai, and Mamoré rivers, present in the main channel and perennial and temporary lakes (Súarez *et al*., 2013; Tondato *et al*., 2013; Severo-Neto *et al*., 2015; Froehlich *et al*., 2017; Gimênes Júnior, Rech, 2022).

Markiana nigripinnis is characterized by teeth with sharp cusps, a single set of teeth in the dentary and two sets in the premaxillary, and an herbivorous feeding habit with a trend to omnivory, and a diet composed of plant origin items, such as roots, fruits, seeds, and fibers (Resende *et al*., 1998; Costa-Pereira *et al*., 2011; Gimênes Júnior, Rech, 2022). Moreover, as they live in tropical environments with an average water temperature ranging from 20 to 26 °C, they are easily raised in aquariums, as they do not require the use of heaters (Géry, 1977; Schäfer, 2009). Another feature that attracts interest from aquarists is the long orange-colored anal fin, a character rarely found in other species of ornamental fish (Schäfer, 2009; Gimênes Júnior, Rech, 2022). Furthermore, it is important to highlight that this is the first record of the reproduction of this species in captivity, and there is no published information on the basic aspects of its biology, even though it is a species exploited by aquarists. Currently, *M. nigripinnis* is included in the list of freshwater fish allowed to be caught, through the Instrução Normativa MAPA/SAP 10/2020 (Ministério da Agricultura, Pecuária e Abastecimento); however, to date, there are no revisions to this list, which highlights the urgency of information to provide subsidies for its maintenance.

During the early ontogeny of fish, several metamorphic processes lead to the differentiation of specific structures, highlighting the importance of these studies in understanding the mortality/survival process and recruitment of species, in addition to providing subsidies for correct identification of larvae in the natural environment (Suiberto *et al*., 2009; Zacardi, Bittencourt, 2017; Stevanato, Ostrensky, 2018). Despite the large number of species in the family Characidae, little is known about the aspects of early ontogeny. According to a survey carried out by Reynalte-Tataje *et al.* (2020), only 2.6% of species have their early development described in the literature, and this number is even lower when considering minute species with little or no commercial interest. Therefore, this study contributed to the knowledge of the early ontogeny of the family Characidae in the Neotropical region, through the description of larvae and juveniles of *M. nigripinnis* bred in captivity, considering morphological, pigmentation and morphometric aspects, as well as analyzing changes in growth patterns, testing the hypothesis of differential development during the species' early ontogeny.

MATERIAL AND METHODS

Sampling sites. *Markiana nigripinnis* adults were collected in the Miranda River basin and transported to the Laboratório de Ictiologia do IMASUL (Instituto de Meio Ambiente do Mato Grosso do Sul), located in the municipality of Campo Grande, state of Mato Grosso do Sul, Brazil, where they were acclimatized to aquarium conditions for about 20 minutes, to reduce the stress caused by management and habitat change (Krishnakumar *et al*., 2020; Jones *et al*., 2022), and later transferred to their respective storage tanks.

For the reproduction process, six sexually mature matrices (two females and four males) were selected. Sexing and determination of maturity stages were carried out through the observation of the presence of bone processes in mature males, where there is the formation of spikes on the anal-fin rays, by a touch analysis of their roughness. These matrices were placed in two polyethylene tanks, 480 liters each, fed daily with flock feed at the end of the day, and exposed to the open air so that in each tank there were two males and one female. Some environmental variables were monitored so that the water temperature remained at an average of 26.0 °C, the water conductivity at 82 $\mu S.cm^{-1}$, slightly acidic pH (6.5), and ammonia (NH^3/NH^{+4}) and nitrogen dioxide (NO_2) indices at 0.00 mg.L and 0.05 mg.L, respectively. In this environment, some macrophytes were inserted to simulate a natural spawning habitat. All tanks were covered with a 0.5 mm mesh screen to control sun exposure and avoid the presence of predators.

The environment was monitored three times a day (early morning, noon and late afternoon), to observe behaviors suggesting spawning, such as the movement of fish close to macrophytes (Andrade, Yasui, 2003; Rotta, 2004). As soon as the presence of eggs in the tanks was observed, matrices were taken and collections began immediately. However, it was not possible to collect eggs at the exact moment of spawning, making it impossible to fully describe this period of development; because of this, it was only possible to collect larvae and juveniles, which are described in this study. Larvae were fed with *Artemia* spp. and juveniles with flock feed.

From November to December 2020, five individuals were collected daily at nine in the morning, whenever possible, for 30 days, which coincided with the end of the species' early development. Individuals were caught with the aid of a 0.5 mm mesh sieve, and in all samples obtained, eugenol (4-allyl-2-methoxyphenol; 0.00005 mL/L) was previously added (according to the CONCEA Euthanasia Practice Guideline; Resolução Normativa MCTI/CONCEA Nº 28, November 13, 2015), as a euthanasia method. Then, the samples were fixed using 4% formalin, buffered with calcium carbonate (1 g $CaCO_3$ in 1000 mL formalin) (Nakatani *et al*., 2001) and transferred to containers labeled with the sample code and collection date. After completing the collections, the material was transported to the Laboratório de Ecologia e Peixes de Água Doce, at the Universidade Federal do Mato Grosso do Sul (UFMS), Brazil, where meristic and morphometric analyses were performed.

Analyses. Individuals were classified according to developmental stages into larval (yolk-sac, preflexion, flexion, and postflexion stages) and juvenile periods, according to Ahlstrom, Moser (1976), modified by Nakatani *et al*. (2001) using a stereomicroscope. Thus, the description of each period or stage was based on the developmental stage and the occurrence of the main morphological events, and illustrations were made using a

camera lucida and a digital camera attached to the stereomicroscope, considering the individuals that best represented the early development. Individuals used in the research were deposited in the Fish Collection of the Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia) of the Universidade Estadual de Maringá (UEM), Maringá, Paraná (NUP 24104).

For morphometric characterization of the early development, morphometric variables (mm) were obtained using the protocol described by Costa *et al*. (2016). For this, a Zeiss Discovery V20 stereomicroscope was used, with image capture to obtain the following variables: total length (TL), standard length (SL), snout length (SnL), eye diameter (ED), head length (HL), head depth (HD), body depth (BD) and distances snout-pectoral fin (SnP), snout-pelvic fin (SnV), snout-dorsal fin (SnD) and snout-anal fin (SnA). For meristic characterization, the total, preanal, and postanal myomeres number were counted whenever possible. Pectoral, pelvic, dorsal, anal, and caudal fin rays were also counted, following Nakatani *et al.* (2001). Larvae were stained with a 1% methylene blue solution (1g methylene blue in 100 mL distilled water) (Lima *et al*., 2021) to facilitate the visualization of some structures, such as the embryonic membrane, myomeres and pelvic fin bud.

For the analyses of body ratios, morphometric variables were expressed as percentages of the standard length and head length throughout development. Body ratios for body depth, head length, and eye diameter were established using criteria suggested by Leis, Trnski (1989).

To test possible ontogenetic variations throughout the development of the species, morphometric variables (response variables) were plotted against the standard and head length (explanatory variables) and their ratios described by different regression models (Kováč *et al*., 1999). Initially, the hypothesis that the development of body ratios is continuous isometric was tested using simple linear regression. With the hypothesis of continuous isometry, alternative hypotheses of gradual allometry (quadratic regression) or discontinuous isometry (piecewise linear regression, which is marked by breakpoints that indicate different growth rates) were tested. The selection of the best model for each morphometric variable relative to body and head size was tested by F-tests (Sokal, Rohlf, 1981). The significance level adopted for the analyses was $p < 0.05$.

RESULTS

Eighty individuals were analyzed, 10 in the yolk-sac stage, 22 in preflexion, 19 in flexion, 20 in postflexion and 9 juveniles. The description of each period and stage is presented below and illustrated in Fig. 1. Results regarding the morphometric and meristic variables are listed in Tab. 1. The main morphological events during the early development of *M. nigripinnis* are summarized in Fig. 2.

Larval development. Yolk-sac larvae (Fig. 1A; Tab. 1) (1st and 2nd day): The standard length ranged from 3.1 to 3.8 mm SL (mean ± SD = 3.5 mm ± 0.3). Early development is altricial, that is, are poorly developed at hatching. Larvae at this stage have an unflexed notochord, a relatively large yolk sac, occupying approximately 1/3 of the body, and an adhesive organ on the top of the head. The mouth and anus are

TABLE 1 | Minimum (Min), maximum (Max), mean (X) values and standard deviation (SD) (mm) for morphometric variables, body relations (%) and meristic counts in larvae and juveniles of *Markiana nigripinnis*. YS = yolk-sac larvae, PF = preflexion stage, FL = flexion stage, FP = postflexion stage, J = juveniles, AF = absent fin, NV = not visible, TL = total length, SL = standard length, SnL = snout length, ED = eye diameter, HL = head length, HD = head depth, BD = body depth, SnP = distances snout-pectoral fin, SnV = snout-pelvic fin, SnD = snout-dorsal fin, and SnA = snout-anal fin.

Variables (mm)	Larval period																Juvenile period			
Stage	YS (n=10)				PF (n=22)				FL (n=19)				FP (n=20)				J (n=9)			
	Min	Max	X	SD	Min	Max	X	SD	Min	Max	X	SD	Min	Max	X	SD	Min	Max	X	SD
TL	3.11	4.10	3.67	0.41	4.13	5.36	4.66	0.36	6.86	9.56	8.12	0.82	9.86	15.82	12.37	1.46	15.28	30.81	23.90	4.38
SL	3.06	3.82	3.48	0.34	3.84	5.11	4.39	0.35	6.06	8.31	7.04	0.67	8.33	12.31	10.06	0.95	12.14	24.25	18.61	3.47
SnL	0.06	0.20	0.17	0.04	0.10	0.23	0.19	0.03	0.34	0.51	0.40	0.05	0.47	0.85	0.61	0.10	0.72	1.81	1.33	0.31
ED	0.16	0.33	0.26	0.06	0.27	0.42	0.35	0.04	0.57	0.85	0.67	0.08	0.82	1.51	1.11	0.17	1.38	2.51	2.06	0.34
HL	0.64	0.76	0.73	0.05	0.82	1.10	0.96	0.09	1.49	1.99	1.68	0.16	2.17	3.61	2.75	0.40	3.22	7.45	5.35	1.23
HD	0.56	0.70	0.64	0.06	0.59	0.94	0.77	0.10	1.12	1.60	1.32	0.17	1.79	3.57	2.47	0.44	2.99	7.16	5.34	1.18
BD	0.72	0.84	0.76	0.04	0.58	0.94	0.72	0.08	1.15	1.86	1.43	0.24	2.04	4.10	2.86	0.49	3.66	8.45	6.57	1.52
SnP	0.76	0.87	0.84	0.04	0.81	1.27	1.07	0.12	1.54	2.35	1.91	0.21	2.29	4.42	3.10	0.53	3.89	8.25	6.18	1.28
SnV	AF	AF	AF	AF	AF	AF	AF	AF	AF	AF	AF	AF	3.77	6.11	4.81	0.56	5.80	12.04	9.18	1.81
SnD	AF	AF	AF	AF	AF	AF	AF	AF	2.74	3.93	3.35	0.32	4.08	6.16	4.88	0.50	6.00	11.08	9.15	1.57
SnA	AF	AF	AF	AF	AF	AF	AF	AF	3.75	4.76	4.27	0.32	4.85	7.32	5.86	0.58	7.02	14.85	11.31	2.30
Relations (%)																				
HD/HL	79.13	92.25	87.81	4.86	69.92	88.49	79.75	5.52	72.80	88.60	78.29	4.78	76.47	99.14	89.54	5.87	92.74	110.46	99.96	6.52
SnL/HL	24.93	26.34	25.77	0.47	11.91	26.47	21.07	3.58	20.55	27.21	23.75	1.68	18.40	24.99	22.20	1.99	21.29	29.11	24.89	2.37
ED/HL	39.57	46.42	42.19	2.53	32.32	41.11	36.18	2.45	37.39	46.30	40.10	2.29	37.09	47.55	40.59	2.50	33.62	43.28	38.95	3.05
HL/SL	17.88	20.14	19.57	0.85	20.11	24.71	21.79	1.16	21.85	25.48	23.89	0.87	23.62	29.84	27.24	1.72	25.55	30.87	28.59	1.92
BD/SL	18.93	22.84	20.46	1.39	13.91	17.96	16.12	1.11	16.96	23.25	20.22	1.72	24.53	33.27	28.23	2.22	30.13	39.17	35.07	3.27
SnP/SL	20.85	22.90	22.35	0.77	21.18	27.35	24.23	1.43	24.26	29.97	27.15	1.52	23.81	36.27	30.66	2.78	30.00	35.16	33.10	1.60
SnV/SL	AF	AF	AF	AF	AF	AF	AF	AF	AF	AF	AF	AF	44.79	54.31	47.74	2.19	47.19	52.23	49.24	1.63
SnD/SL	AF	AF	AF	AF	AF	AF	AF	AF	43.20	50.43	47.59	1.71	45.77	52.03	48.50	1.68	44.88	52.84	49.36	2.85
SnA/SL	AF	AF	AF	AF	AF	AF	AF	AF	56.45	64.04	59.76	1.74	56.27	59.46	58.28	0.79	57.86	63.15	60.59	1.66
Rays																				
Pectoral	NV	NV	NV	NV	NV	NV	NV	NV	NV	NV	NV	NV	NV	NV	NV	NV	11.00	13.00	12.22	0.83
Ventral	AF	AF	AF	AF	AF	AF	AF	AF	AF	AF	AF	AF	5.00	7.00	6.00	0.53	6.00	7.00	6.44	0.53
Dorsal	AF	AF	AF	AF	AF	AF	AF	AF	8.00	10.00	9.00	0.76	10.00	11.00	10.65	0.49	11.00	12.00	11.11	0.33
Anal	AF	AF	AF	AF	AF	AF	AF	AF	AF	AF	AF	AF	30.00	47.00	41.94	4.61	43.00	47.00	44.67	1.50
Caudal	AF	AF	AF	AF	AF	AF	AF	AF	16.00	21.00	19.13	1.64	19.00	24.00	20.85	1.53	20.00	27.00	23.67	2.40
Myomers																				
Pre-anal	16.00	18.00	16.70	0.67	16.00	17.00	16.84	0.37	17.00	20.00	18.11	0.66	19.00	22.00	20.00	1.08	NV	NV	NV	NV
Postanal	18.00	19.00	18.70	0.48	19.00	20.00	19.37	0.50	20.00	24.00	21.58	1.30	23.00	27.00	24.90	1.45	NV	NV	NV	NV
Total	34.00	37.00	35.40	0.84	35.00	37.00	36.21	0.54	37.00	42.00	39.68	1.60	42.00	49.00	44.90	2.36	NV	NV	NV	NV

closed and not functional. The anal opening is located over the middle region of the body. The operculum is not formed, as well as the nostrils. The swim bladder is visible but not inflated. Eyes are spherical and show some pigmentation at about 3.6 mm SL. Also, at 3.6 mm SL, some dendritic chromatophores can be seen in the ventral region and the distal portion of the yolk sac. The finfold surrounds the body from the upper medial region to the posterior end of the yolk sac. The pectoral fin bud is visible at about 3.8 mm SL. Total number of myomeres range from 34 to 37 (16 to 18 pre- and 18 to 19 postanal).

Preflexion stage (Fig. 1B; Tab. 1) (days 2–11): The standard length ranged from 3.8 to 5.1 mm SL (mean ± SD = 4.4 mm ± 0.4). At this stage, the notochord is straight and visible. The mouth is open and in the terminal position, the yolk sac is still present, and the adhesive organ is fully absorbed; the eyes are spherical and fully pigmented. The intestine is functional (presence of *Artemia* spp., in 4.3 mm SL larvae), and the anal opening is located over the middle region of the body. The inflated swim bladder is visible through transparency. Pigmentation is composed of punctate chromatophores irregularly distributed in the upper part of the head, in the body and the region of the digestive tract. At this stage, it is already possible to visualize the formation of the operculum and the nostrils, which are simple. The finfold is visible surrounding the entire body, as well as the pectoral bud. Total number of myomeres range from 35 to 37, with 16–17 pre- and 19–20 postanal.

Flexion stage (Figs. 1C–D; Tab. 1) (days 11–22): The standard length of larvae varies between 6.1 and 8.3 mm SL (mean ± SD = 7.0 mm ± 0.7). This stage is characterized by the flexion of the notochord final section, which remains visible through transparency. The mouth is terminal, and the eyes remain spherical. At this stage, the yolk sac is fully absorbed. The swim bladder and the operculum are visible; the nostrils are still simple. Pigmentation becomes continuous, with punctate chromatophores mainly on the top of the head and around the mouth and irregularly distributed throughout the body. It is also possible to see pigment lines in the dorsal, ventral, and lateral regions. At the base of the caudal fin, these chromatophores are distributed along the rays and initiate the formation of a macula. At this stage, the finfold still involves part of the body; it is possible to visualize the hypural boards and the appearance of the first rays of the caudal and dorsal fin at 7.3 mm and 8.3 mm SL, respectively, in addition to the delineation of the anal fin. The pectoral bud remains without rays. The myomere total numbers vary from 37 to 42 (17 to 20 pre- and 20 to 24 postanal).

Postflexion stage (Figs. 1E–F; Tab. 1) (days 22–30): The standard length ranges from 8.3 to 12.3 mm SL (mean ± SD = 10.1 mm ± 0.9). The notochord and swim bladder are visible only at the beginning of the stage, due to the loss of transparency with the development of the musculature. The mouth remains terminal. The nostril becomes double. Pigmentation occurs from dendritic and punctate chromatophores, irregularly distributed throughout the larval body, but also forming longitudinal lines in the dorsal, ventral, and lateral regions, with a greater concentration on the top of the head and around the mouth, as well as in the peduncle and at the base of the caudal fin, where there is the formation of a conspicuous macula. The finfold is almost

completely absorbed, with a remnant observed ventrally, close to the anal region. The pelvic fin bud is visible, and the first rays are formed from 10.4 mm SL. The pectoral fin is the last to develop, not showing a complete formation of rays at this stage; there is also the formation of the adipose fin (11 mm SL), and the caudal fin is forked. The sequence of fin formation, including ray segmentation, is caudal, dorsal, anal, pelvic, and pectoral. The total number of myomeres varies from 42 to 49 (17 to 22 pre- and 23 to 27 postanal); however, due to the development of the musculature, the visualization of myomeres starts to be compromised. The ranges of the number of rays at this stage are caudal = 19–24, dorsal = 10–11, anal = 30–47, and pelvic = 5–7.

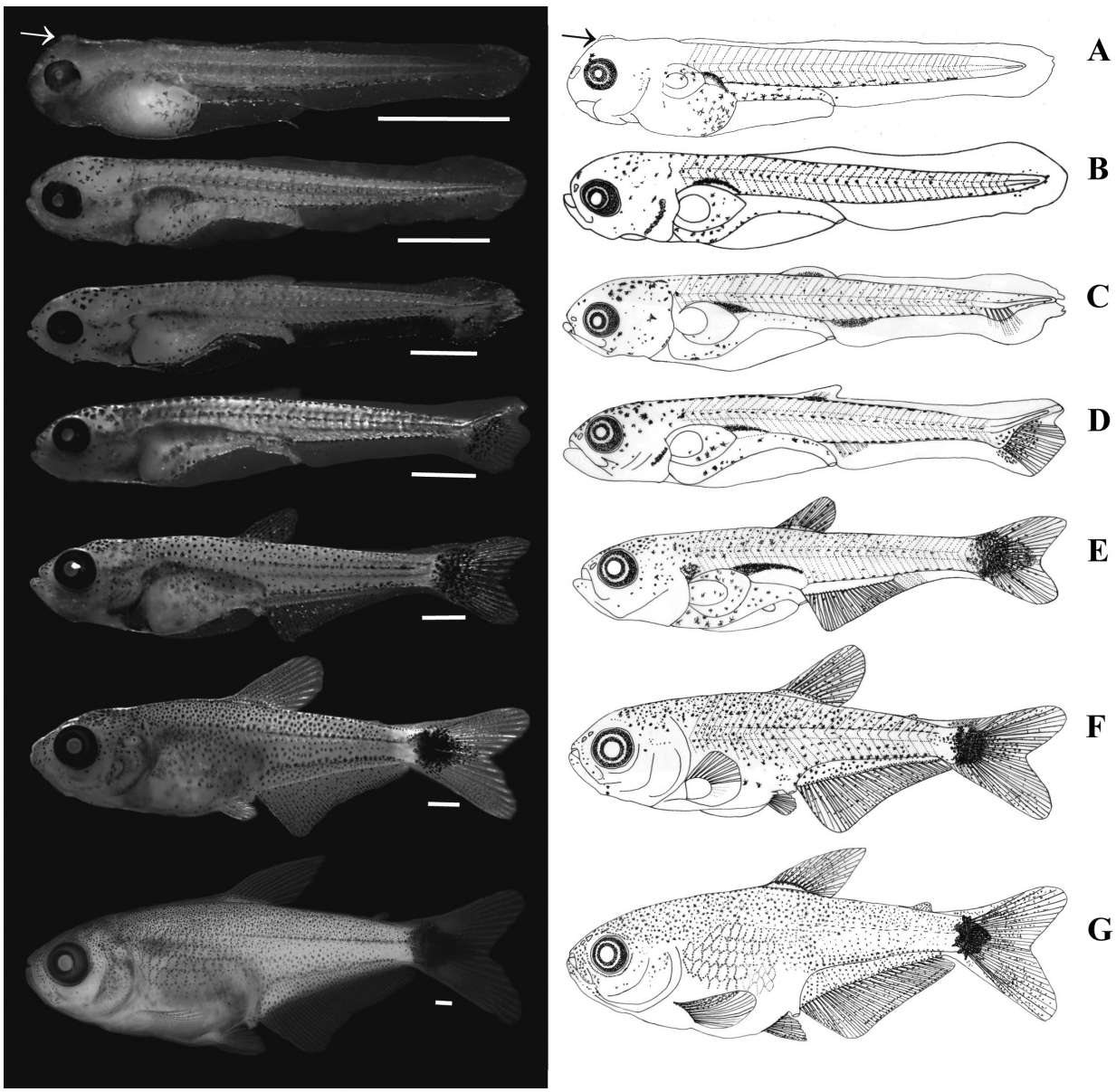

FIGURE 1 | Early development of *Markiana nigripinnis*; **A.** Yolk-sac larvae (3.6 mm SL) (arrow indicates adhesive organ), **B.** Preflexion larvae (5.1 mm SL), **C.** Early flexion larvae (7 mm SL), **D.** Flexion larvae (8.3 mm SL), **E.** Early postflexion larvae (12.3 mm SL), **F.** Postflexion larvae (17 mm SL), **G.** Juvenile (17.6 mm SL); scale bars = 1 mm.

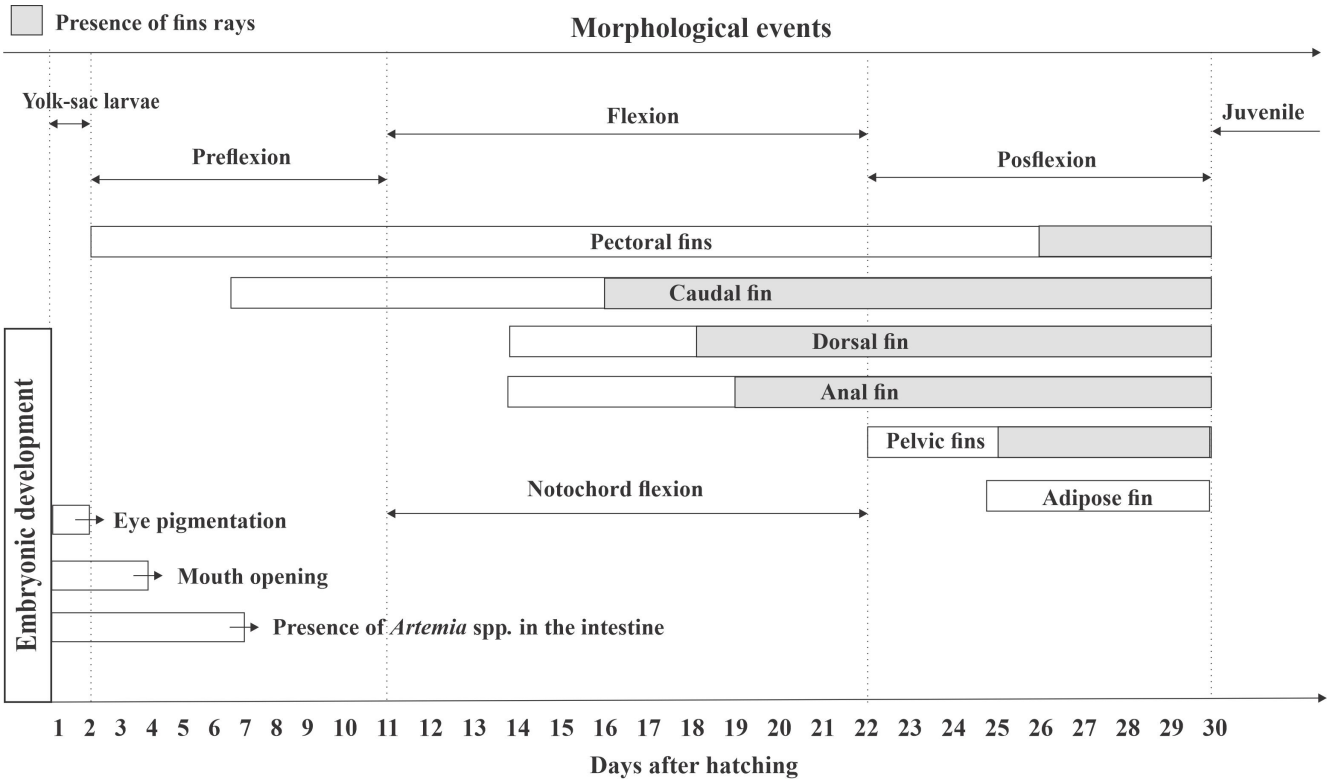

FIGURE 2 | Morphological events summary observed during the early development of *Markiana nigripinnis*.

Juvenile development. This period starts on the 30th day after the hatching of the eggs. Nine individuals with standard length ranging from 12.1 to 24.2 mm SL (mean ± SD = 18.6 mm ± 3.5) were analyzed (Fig. 1G; Tab. 1). Individuals have a terminal mouth, fully pigmented eyes, double nostrils, median anal in the middle region of the body, and scales distributed throughout the body, mainly in the region of the digestive tract. Pigmentation spreads throughout the body, concentrated in the upper portion. In the ventral portion, pigments become more disorganized and random. At the top of the head and the base of the caudal fin, there are the highest concentrations of dendritic and punctate chromatophores, a conspicuous macule between the peduncle and the base of the caudal fin rays. Small chromatophores can be seen between the dorsal, anal, and caudal fin rays, but in the latter, two regions without pigmentation are observed in the middle of the lower and upper rays. All fins are completely formed, and the numbers of rays are caudal = 20–27(dorsal lobe = 9–12, ventral lobe = 11–15), dorsal = 11–12, anal = 43–47, pelvic = 6–7 and pectoral = 11–13.

Morphometric relations. Morphometric variables, as a function of standard length, showed changes in their proportions throughout ontogeny. Regarding body height, it ranged from long to moderate, as well as head length, from small to moderate. The pectoral, pelvic, and dorsal snout-fin distances also increased in proportion throughout development, except the snout-anal-fin distance, which maintained a similar ratio during development (Tab. 1).

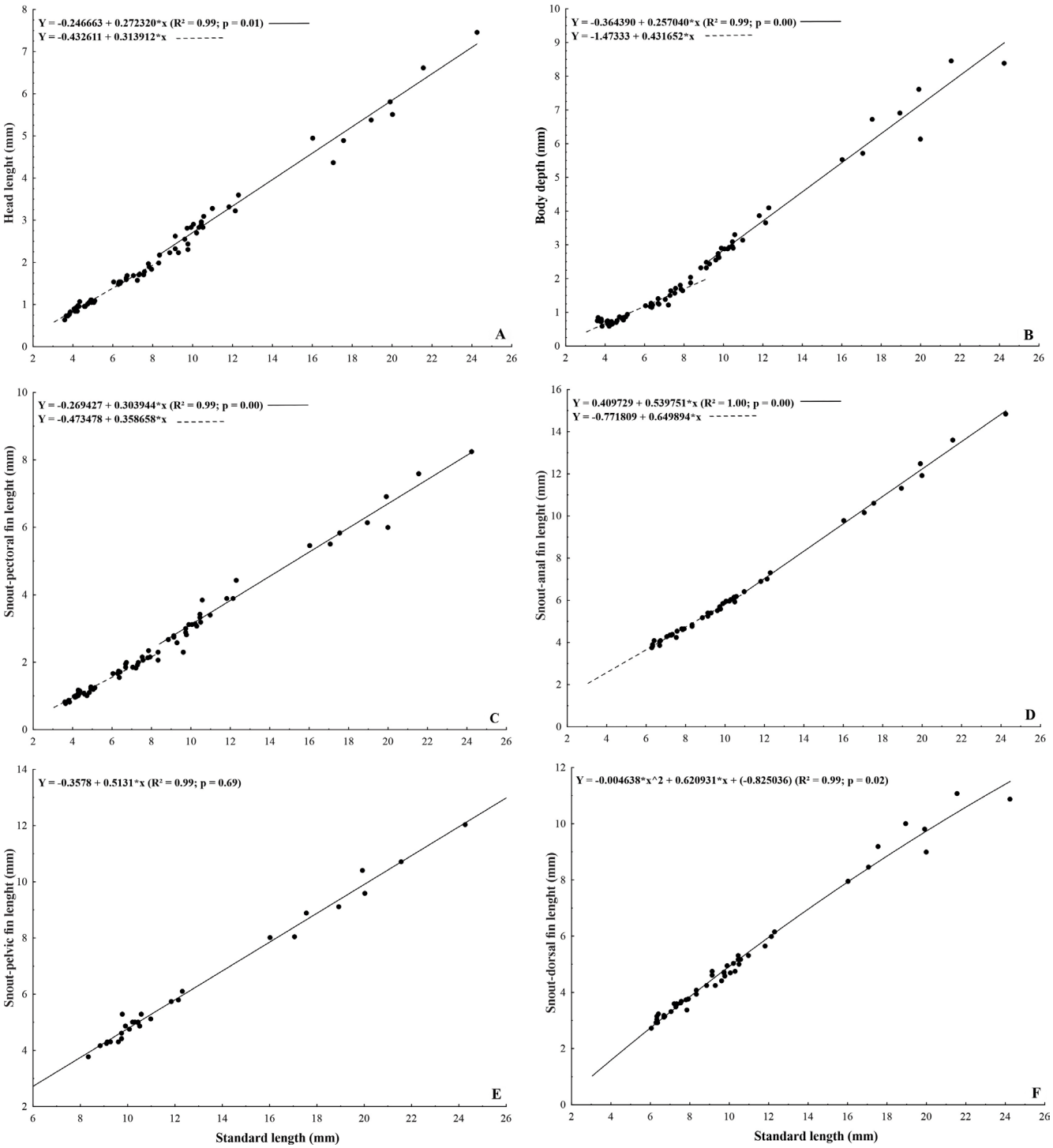

FIGURE 3 | Morphometric relationships (mm) between **A.** Standard length and head length, **B.** Standard length and body depth, **C.** Standard length and snout-pectoral fin length, **D.** Standard length and snout-anal fin length, **E.** Standard length and snout-pelvic fin length, **F.** Standard length and snout-dorsal fin length during the early development of *Markiana nigripinnis*.

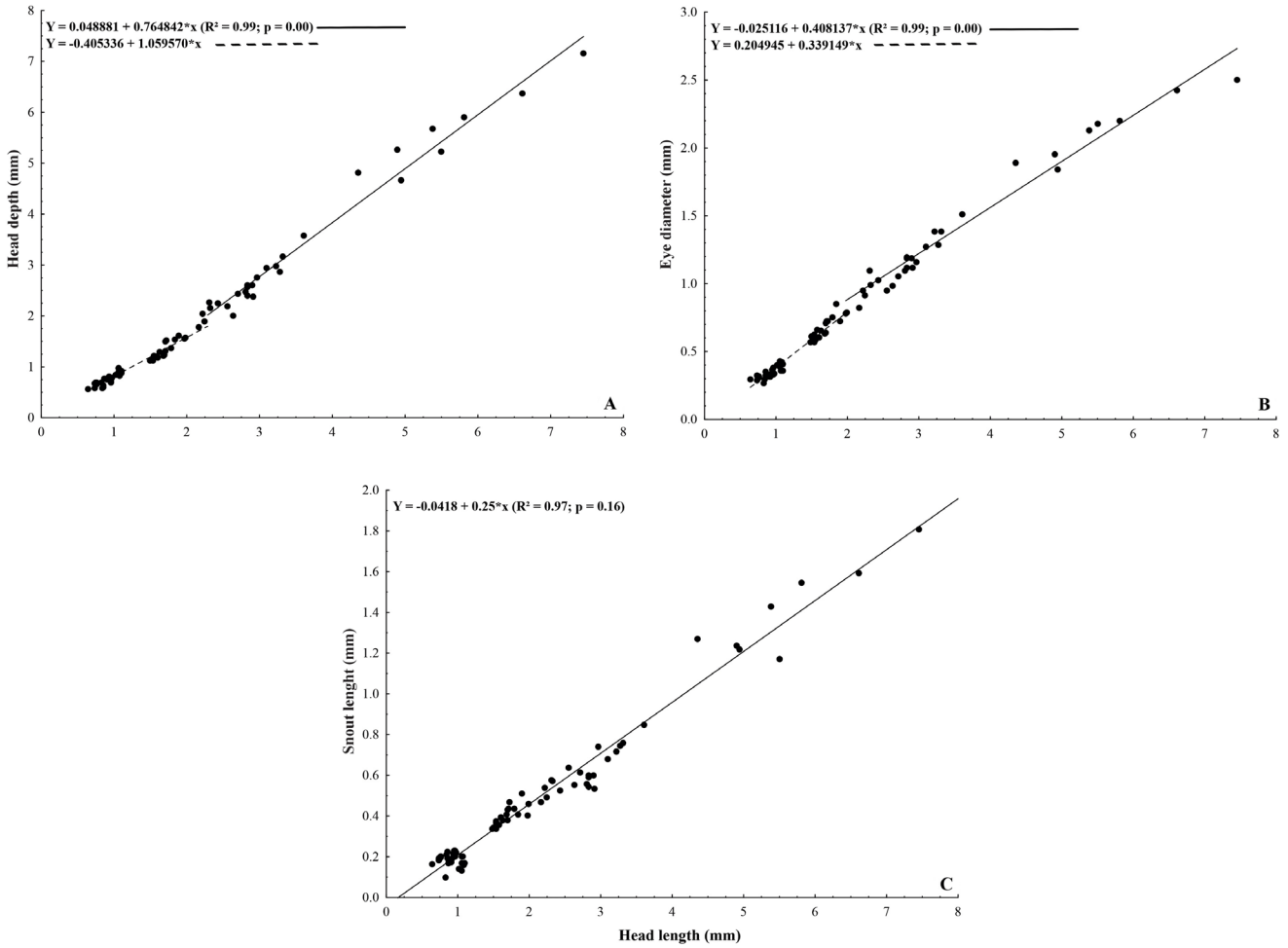

FIGURE 4 | Morphometric relationships (mm) between **A.** Head length and head depth, **B.** Head length and eye diameter, **C.** Head length and snout length during the early development of *Markiana nigripinnis*.

When we analyzed the ratios as a function of head length, we observed that the eye diameter remained large throughout development. For head height, we observed a considerable variation during development, unlike snout length (Tab. 1).

Growth analyses. Regarding the growth patterns between the morphometric variables, we verified that the HL, the BD, SnP, and SnA, all related to the SL (Tab. 2; Fig. 3A–D), were better represented by the piecewise linear regression model, as well as HD and ED, both related to HL (Tab. 2; Figs. 4A–B). Thus, growth started similarly; however, after a certain size of the body or head (breakpoint), there was a sudden change in this pattern.

Snout length (SnL) relative to HL (Tab. 2; Fig. 4C) and SnV related to SL (Tab. 2; Fig. 3E) were best represented by linear regression, while the SnD related to SL (Tab. 2; Fig. 3F) was the only one best represented by a quadratic regression model, with negative allometric growth (Tab. 2).

TABLE 2 | Linear, quadratic, and piecewise regression statistics for the morphometric variables relative to the head length and standard length of larvae and juvenile of *Markiana nigripinnis*. R^2= coefficient of determination, L = linear regression, Q = quadratic regression, S = piecewise regression, BM = best model, BP = breakpoint, N = number of individuals analyzed; bold values presented $p < 0.05$.

Relation	R^2 L	R^2S	R^2 Q	F Q/L	p	F S/Q	p	F S/L	p	BM	BP	N
SnL/HL	0.97	0.97	0.97	1.97	0.16	1.62	0.21	1.80	0.18	L	0.49	76
ED/HL	0.98	0.99	0.99	70.11	0.00	-13.54	0.00	21.20	0.00	S	0.83	76
HD/HL	0.99	0.99	0.99	3.52	0.06	22.25	0.00	13.40	0.00	S	1.89	76
HL/SL	0.99	0.99	0.99	2.12	0.15	7.13	0.01	4.72	0.03	S	2.12	76
BD/SL	0.98	0.99	0.98	13.62	0.00	38.01	0.00	29.31	0.00	S	2.16	76
SnP/SL	0.99	0.99	0.99	0.39	0.53	18.26	0.00	9.37	0.00	S	2.40	76
SnV/SL	0.99	0.99	0.99	0.16	0.69	0.13	0.72	0.14	0.71	L	6.16	29
SnD/SL	0.98	0.99	0.99	6.14	0.02	-2.28	0.14	1.70	0.20	Q	5.07	48
SnA/SL	1.00	1.00	1.00	26.49	0.00	5.98	0.02	17.80	0.00	S	6.33	46

DISCUSSION

In this study, we described the early development of the tetra *M. nigripinnis*, a small characid occurring in the Paraguai River basin. Thus, like other minute species of this family (Galuch *et al*., 2003; Anjos, Anjos, 2006; Çelik *et al*., 2012; Walter, 2012; Mattox *et al*., 2014; Park *et al*., 2014; Marinho, 2017; Santos *et al*., 2017), the species has an altricial development, with poorly developed larvae at hatching, becoming more robust as it develops, and juveniles with morphological characteristics similar to adults.

The yolk-sac larval stage was considered a very rapid period (maximum 48 hours). Larvae initially had eyes without pigmentation, but before the end of the stage, they were partially pigmented, similar to that found by Oliveira *et al.* (2012) when evaluating a Bryconidae species, *Brycon hilarii* (Valenciennes, 1850). Visual sensitivity is extremely necessary since the gradual development of this sensory system soon after hatching has a direct impact on the ability of individuals to perceive a threat or food, which is a determinant of the vertical distribution of larvae (Fuiman, 1983). Additionally, features such as a notochord visible by transparency, an intestine with medium length and embryonic membrane (finfold) surrounding the body from the upper medial region to the posterior end of the yolk sac, are characteristics found in another Characidae, *A. lacustris* (Santos *et al*., 2020). At this stage, we also observed the emergence of the pectoral fin bud, as for the Characidae, *Paracheirodon innesi* (Myers, 1936), *Moenkhausia pittieri* Eigenmann, 1920 (Marinho, 2017) and *A. lacustris* (Santos *et al*., 2020).

From the moment the digestive system becomes functional and the larvae acquire the ability to swim, the shift from endogenous to exogenous feeding occurs, since after the depletion of the yolk sac, these individuals can survive only for a short period (Fuiman, 1983; Stevanato, Ostrensky, 2018). For *M. nigripinnis*, the intestine becomes functional at the preflexion stage, where the presence of *Artemia* spp. was observed, but at this stage there are still remnants of yolk, which is fully absorbed only in flexion,

thus enabling this complete transition. The position of the mouth is related to the type of food acquisition; in *M. nigripinnis*, the mouth is terminal, without changes during ontogeny, indicating that these larvae feed directly in the water column (Bemvenuti, Fischer, 2010). However, this is a characteristic that varies within the family and subfamily, as Galuch *et al*. (2003) and Santos *et al.* (2020) described for the Stervadiinae *Piabarcus stramineus* (Eigenmann, 1908) and Characidae *A. lacustris*, respectively, an initially subterminal mouth with a shift to a terminal during early ontogeny, possibly related to changes in behavior and/or food items.

During larval development, individuals become able, for the first time, to move in space, interact with biotic and abiotic components and actively influence their survival in various ways, due to the development of their musculature and fins (Fuiman, 1983). Fins of fish are intended to provide propulsion, stability, maneuver and defense, and their shape and position are regulated by the principles of hydrodynamics, based on different body shapes and swimming speeds (Bemvenuti, Fischer, 2010). Generally, Characiformes have the development fins in the following order: caudal, dorsal, anal, pelvic, and pectoral (Nakatani *et al*., 2001; Galuch *et al*., 2003), similar to that observed for *M. nigripinnis*. The adipose fin appears at the postflexion, as described for the Stervadiinae, *P. stramineus* (Galuch *et al*., 2003), the Characidae, *Moenkausia* cf. *gracilima* (Santos *et al*., 2017), and *Paracheirodon innesi* (Marinho, 2017). As there is a transition from undulating locomotion to caudal locomotion, both the dorsal located posteriorly and the adipose remain symmetrically opposed to the anal and function as stabilizing rudders, while the pelvic and pectoral fins, which appear posteriorly, serve to control the vertical course during movement locomotion (Breda *et al*., 2005; Bemvenuti, Fischer, 2010).

Given the importance of characterizing pigmentation for identifying larvae and considering that each species has a distinct pattern, with the size and location of melanophores being genetically determined (Nakatani *et al*., 2001; Andrade *et al*., 2014), there was an increasing pigmentation, with pattern variation throughout the development of *M. nigripinnis*. The small amount or absence of pigmentation right after hatching shows a pelagic behavior; however, the intensification of these chromatophores during development occurs when individuals begin to explore littoral zones (*e.g*., covered areas by macrophytes) (Nakatani, 1997; Sanches *et al*., 2001; Iquematsu *et al*., 2022), as expected for the analyzed species.

At the yolk-sac stage, the presence of dendritic chromatophores in the distal part of the yolk sac was evident, as observed for *A. lascustris* (Stevanato, Ostrensky, 2018; Santos *et al*., 2020). From the flexion stage, there is the development of the macula in the caudal fin, which is characteristic of the species, and an increase of pigments in the body, forming longitudinal lines, in addition to the generalized distribution throughout the body. These results demonstrate a pigmentation with a different pattern than that observed in Stevardiinae, *Bryconamericus iheringii* (Boulenger, 1887), whose pigmentation is present in a few dendritic clusters in the head region, ventral region and above the anal fin, and anteriorly to the caudal fin (Orsi *et al*., 2016). This was also observed by Galuch *et al.* (2003) for the species *P. stramineus* in a different pattern, which presents a pigmentation concentrated on the top of the head and diffused throughout the body, indicating that the pigmentation observed in *M. nigripinnis*, including the macula on the caudal peduncle, are characteristics that aid in identification within the subfamily.

Another important feature for identification is the myomere number (Snyder,

1979). When compared with other species, *M. nigripinnis* presents a value of 34 to 49 myomeres (16 to 23 pre- and 18 to 27 postanal), against 35 to 40 (16 to 21 pre- and 17 to 22 postanal) *P. stramineus* (Galuch *et al*., 2003), 28 to 40 (13 to 20 pre- and 14 to 18 postanal) *A. lacustris* (Santos *et al*., 2020) and 32 to 37 (17 to 19 pre- and 15–18 postanal) for *Astyanax altiparanae* Garutti & Britski, 2000 (synonym of *A. lacustris*) (Nakatani *et al*., 2001), all characids, and can be considered in the identification of larvae of this species.

In addition to myomeres, the number of fin rays may allow the distinction of orders or families (Ré, 1999), in which the anal fin is the one that most distinguishes *M. nigripinnis* from other Characidae, as it presents a variation of 43–47 rays, against 20 to 23 of *P. stramineus* (Galuch *et al*., 2003), 21–23 of *Moenkhausia* cf. *gracilima* (Santos *et al*., 2017), 17–23 of *Aphyocharax* cf. *anisitsi* (Nakatani *et al*., 2001), 20–24 of *H. marginatus* (Nakatani *et al*., 2001), 27–29 of *A. lacustris* (Santos *et al*., 2020). With this, these characters can be parameters used to assist in the identification of the larvae of this species in the natural environment.

Markiana nigripinnis larvae showed morphometric variations throughout their ontogeny, with a long to moderate body, and small to moderate head, changes like those found in *P. stramineus* (Nakatani *et al*., 2001). Concerning eye diameter, our results showed large eyes in all stages, corroborating what was observed for *A. lacustris* (Santos *et al*., 2020) and contrasting the increase in eye size (moderate to large) in the larval development of *P. stramineus* (Nakatani *et al*., 2001). This suggests that during ontogeny there is no change in the type of feeding since Santos *et al*. (2017) also described a moderate to large diameter during the development of *Moenkausia* cf. *gracilima*, suggesting a change in feeding, a fact that is common during the early development of some species. Large eyes at late development (postflexion) have also been reported for Stervadiinae, *B. iheringii* (Orsi *et al*., 2016).

These results, along with the snout length and high depth of the head during development, suggest that the increment and growth of structures led to the need for the expansion of the body and head to moderate. Taguti *et al*. (2009) examined the early development of *Pyrrhulina australis* Eigenmann & Kennedy, 1903 and suggested that the change in the body from long to moderate is a consequence of the development of the musculature and the digestive tract, being linked to environmental conditions, such as competition and food capture that would accelerate this body development. Furthermore, head growth is probably due to the formation of the brain part of the larvae, which leads to the diversification of motor and sensory skills and the development of the gill apparatus and the onset of exogenous feeding (Blaxter, 1988). Thus, our results indicate that the characteristics are related to the strategy for survival in their habitat during their development, showing changes in morphology, large eyes, and pigmented body to search for food while camouflaging below marginal aquatic plants.

The species growth pattern may be related to a greater morphophysiological demand (Oliveira *et al*., 2020). The growth models observed between the snout length and head length, and pre-pelvic distance and standard-length relationships suggest negative allometric growth ($b < 1$), as well as the quadratic regression found for the snout-dorsal fin distance and standard length (Oliveira *et al*., 2020; Santos *et al*., 2020). Variables that present abrupt growth, that is, with breakpoints, are considered significant if associated with some morphological, physiological and/or survival event (Kováč *et al*., 1999; Taguti *et al*., 2009; Santos *et al*., 2017). For *M. nigripinnis*, eye diameter, head depth, body depth,

and snout-pectoral fin and snout-anal fin distances showed this type of relationship, suggesting that in this interval, during the flexion stage, most remodeling of the external form of the body occurs, in addition to the onset of exogenous feeding (Taguti *et al*., 2009; Santos *et al*., 2017), which indicates the period of major metamorphosis of the species. It is possible to verify the presence of breakpoints for similar species, such as *Moenkausia* cf. *gracilima* (Santos *et al*., 2017) that has a similar development when we observe the eye diameter and snout-pectoral fin, as well as for *Astyanax lacustris* (Santos *et al*., 2020) where it is possible to verify the size related to head depth and body depth.

In summary, this study is a pioneer in the description of the early ontogeny of a small characid, through semi-natural breeding, with matrices from the Paraguai River basin. Our findings contribute to information about the early development of the tetra *M. nigripinnis*, helping reduce the existing knowledge gap on the life history of fish in the Neotropical region, especially of the family Characidae. Furthermore, it contributes information that may support aquaculture production and conservation measures for the region.

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AUTHORS' CONTRIBUTION

Mateus Babichi Veiga de Souza: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing-original draft, Writing-review and editing.

Karina Keyla Tondato-Carvalho: Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Writing-review and editing.

Heriberto Gimênes Junior: Funding acquisition, Investigation, Methodology, Project administration, Writing-review and editing.

Andréa Bialetzki: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Writing-review and editing.

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ETHICAL STATEMENT

Technical manifestation/GPF/IMASUL N°014/2015. In all samples obtained, eugenol (4-allyl-2-methoxyphenol; 0.00005 mL/L) was previously added (according to the CONCEA Euthanasia Practice Guideline; Resolução Normativa MCTI/CONCEA No 28, November 13, 2015), as a euthanasia method.

COMPETING INTERESTS

The author declares no competing interests.

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