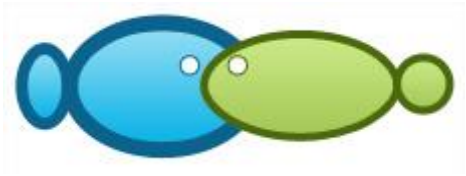

Practical differentiation between species and sexes of juvenile tropical ornamental fish in the genus *Nematobrycon* and new localities for the species

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Abstract. The well-known tropical ornamental fishes, 'the emperor tetras', of the genus *Nematobrycon*, are two distinct, sexually dimorphic species that can be identified within 3 to 4 months of age. Male Emperor Tetras have blue eyes, while Rainbow Tetras have red eyes. Males of both species also possess trident-shaped caudal fins. Females of both species have either 'pale gold' or 'metallic green' eyes, as previously described in the literature. Species identification was confirmed through molecular barcoding, and gonad histology results matched 100% with sexual dimorphism findings. The geographic range now includes the entire river for both species, with the Emperor Tetra found in the Río San Juan watershed and the Rainbow Tetra in the Río Calima watershed. Due to the geographic closeness of the river watersheds, this can result in hybrid speciation.
Key Words: emperor tetra, rainbow tetra, geographical range, taxonomy.

Introduction. Certainly, some of the most beautiful tropical ornamental fish species are the tetra fishes (family Characidae), especially the emperor tetra, *Nematobrycon palmeri*, and its sister species, the rainbow tetra, *Nematobrycon lacortei*. They appeal to a broad audience, including hobby aquarists, as well as in popular aquarium magazines and online sources. Although the emperor tetra was first described by Eigenmann in 1911, and the rainbow tetra by Weitzman & Fink in 1971, both were only introduced to the aquarium hobby by Myers in 1953. His descriptions still relied on preserved museum specimens, with their beauty attributed to the dark coloration patterns on the preserved bodies (Myers 1953). The brief history of the discovery and description of live specimens were not provided until Kyburz (1960 & 1961), Vorderwinkler (1960), and Weitzman & Fink (1971).

Our perspective, similar to that of aquarium enthusiasts, has wondered why these two species, despite being stunningly beautiful and easily recognized in the aquarium trade, are not as widely sought after as other tetras such as the neon (*Paracheirodon innesi*), cardinal (*P. axelrodi*), and rummynose (*Hemigrammus rhodostomus*), among others. Today, as in the past, both species are mainly collected from the wild for the ornamental fish trade, with only a few references mentioning limited production for commercial purposes by hobbyists or artisanal aquaculturists; also, there are a few producers of 'emperor tetras', at the artisanal and small-scale level, mainly in Southeast Asia and Europe.

The geographic locations of the emperor tetra were described as small creeks, backwaters, and tributaries flowing into the headwaters of the San Juan and Atrato rivers in Colombia's Chocó region on the Pacific coastal lowlands (Eigenmann 1911; Myers 1953; Kyburz 1960 & 1961; Axelrod 1971; Weitzman & Fink 1971). Similar riparian areas and aquatic habitats were also described for the rainbow tetra; however, the species has only been recorded in the upper watershed of the Río Calima, which is a tributary of the lower Río San Juan (Kyburz 1961; Weitzman & Fink 1971). Therefore, both of these species are truly tropical fish and are endemic to a very narrow range of coastal watersheds. As Myers (1953) and Kyburz (1961) pointed out, "the *Nematobrycon* country is not easy to reach", and this remains true today. The Pacific coastal lowlands in Colombia remain primarily geographically isolated and culturally unchanged, with a mostly socio-economically disadvantaged population facing complex public safety challenges.

Like many other aquarium fish collected from the wild, they are usually caught at remote fishing sites, and their exact locations are often not documented or reported. Additionally, many of the original species descriptions are based on old museum collections with unverified site reports. Therefore, one objective of this study was to report new site localities for the species. This information is crucial for conservation and management efforts. Their narrow distribution makes them especially vulnerable to natural disturbances, human impact, and potentially lower genetic diversity. Also, because their ranges are geographically close, this can even lead to hybrid speciation.

A second objective of the study was to provide a practical method for distinguishing between the species and sexes of juvenile emperor and rainbow tetras. Both species are mostly caught as juveniles at remote fishing sites and can remain in fish camps for days or even weeks. They are only later classified as being a batch or lot of 'emperor tetras' when a few dimorphic individuals are identified in the caught group, as well as by the basin or region where they were caught. For trade and marketing purposes, species identification primarily relies on original descriptions found in scientific articles and popular hobbyist magazines. These descriptions emphasize the distinctive 'square-finned' body shape of the 'tetra' fishes. Those in the *Nematobrycon* genus are notable because they lack an adipose fin, and the middle rays of the caudal fin in males are elongated, forming a three-forked tail (Eigenmann 1911; Myers 1953; Kyburz 1960; Weitzman & Fink 1971). Articles in aquarium magazines, books, and informational websites, also describe the overall appearance of males, noting they have brighter coloration on the body than females, with blue eyes in male emperor tetras and red in rainbow tetras; in other individuals of both species (probably females), eyes are described as having 'pale gold' or 'metallic green' eyes (Kyburz 1960; Axelrod 1971; Weitzman & Fink 1971; Aqualog 2016; Wikipedia 2025). Although the literature consistently discusses both males and females of either species, no method was found to confirm their biological sex.

Therefore, the current method of distinguishing juvenile emperor and rainbow tetras is impractical, which could lead to uncertain ecological, scientific, and economic risks. In this study, we provide histological confirmation of the sex for both species, based on microscopic examination of gonadal tissue samples from individual fish displaying different dimorphic traits of the two species. Additionally, we report two new locations that expand the geographical range of the emperor and rainbow tetra species.

Material and Method. Fish believed to be emperor and rainbow tetras were collected by a trusted fisherman from two different river locations in mid-year 2024: the lower Río San Juan in the Chocó department and the lower Río Calima in the Valle del Cauca department, both in Colombia. Most of the fish were small in size and had indistinct features, making it difficult to identify them as known species. Collection sites were chosen based on local fishing lore about known fishing grounds around rural communities.

Although similar in size and characterized by the 'tetra body' shape, a moderate number of larger individuals from fish groups in different locations showed differences in their eye and body colors. Fish from the Río San Juan had blue-colored eyes, while those

from the Río Calima had red-colored eyes. Also (regardless of river drainage), only fish with blue or red eyes exhibited the elongated ray in the caudal fin that formed a trident shape. All remaining individuals from either drainage had pale yellow-green eyes. To determine whether individuals from the different drainages were of the same or different species and sexes, the two groups were kept in separate holding tanks for at least six months. The tanks were commercially available plastic containers used for storing drinking water, with a nominal capacity of 1,000 liters (a tank diameter of 150 cm and a water-filled height of 48 cm). They were equipped with aeration (Alita Industries) and filtration systems (foam pad attached to a 3000 LPH submersible powerhead pump for controlling physical, chemical, and biological water quality parameters; XpertMatic, SainSmart). Tanks were exposed to a natural 12-hour light and 12-hour dark photoperiod, and 15% of the water volume was exchanged weekly.

Fish were weighed (0.01 g) and measured (0.1 cm) at the start (one week after capture) and at the end of four-months of a six-month growout period. They were hand-fed daily to satiation with a pelleted commercial diet (150-360 micrometers, Otohime A2, B1). Data were analyzed using descriptive statistics and a t-test at a 95% confidence level, with a computer and software Excel (ver. 22032, Microsoft). To ensure proper species identification, molecular-genetic barcoding was employed. Muscle tissue samples were taken from the small (n = 15) and dimorphic individuals (n = 15) from the two river drainages. The samples were preserved in RNAlater (Ambion-Invitrogen) and sent for analysis to an independent laboratory certified in molecular diagnostics (Applied Food Technologies). Additionally, during the six-month growing period, a total of 60 individuals (n = 30 from each river group) were periodically selected to determine whether dimorphic differences in eye color and trident shape of the caudal fin were associated with their biological sex. For histology, gonad tissue samples were dissected from fixed specimens (in 10% neutral buffered formalin), placed in tissue cassettes, embedded in paraffin blocks, sectioned, and stained with hematoxylin and eosin (H&E) for later analysis under a microscope.

Results and Discussion. One of the main goals of this study is to support the development of aquaculture for the two sister species of ornamental 'emperor tetra'. This aims to aid in their conservation in the wild and serve as an alternative farming system for income generation, thereby enhancing food security and socio-economic stability in the region. Since most aquarium ornamental fish are small, they are typically measured or reported only by length in the scientific literature. This study provided an opportunity to sample juvenile 'emperor tetras' from populations in the wild that consisted of two species and to report their lengths and weights (Table 1). It also allowed us to collect data on fish pack counts and fishermen's prices overall (Table 1). Such information is crucial not only for resource management but especially for small-scale integrated fish farming systems, which depend on this data for system design, estimating loading rates (which influence water quality and fish productivity), and understanding market dynamics (Timmons et al 2009).

Table 1

The marketable size of 'emperor tetras' (total length and weight) at first capture, prices, and number of individuals in a shipping box typically sold wholesale

Species	*Length (cm)*	*Weight (gram)*	*Price (USD)*	*Box (# ind.)*
'Emperor tetra'	2.5±0.3	0.223±0.111	0.10-0.12	400-600

As juveniles, 'emperor tetras' are typically commercialized at a total length of between 2 and 3 centimeters, corresponding to a live weight of 0.223±0.111 g (Table 1). Based on size estimations by the fishermen, at these sizes, juvenile fish are approximately one month in age. At these sizes, it is difficult to distinguish the distinctive characteristics of the species, such as eye color and/or elongated caudal tails; only individuals with "expert eyes" can easily detect these particularities.

The bodies of the smaller fish were silver-yellowish in coloration, as well as their eyes. After a few weeks in culture, a faint dark line became visible laterally along the middle of the body in all individuals. However, after a two- to three-month grow-out period, dimorphism between individuals started to become apparent. The color of the eyes was first observed, followed by elongation of a few rays in the middle of the caudal fin, visible in approximately half of the individuals. Of the fish with elongated rays in the middle of the caudal fin, some had blue eyes while others had red eyes. The other half of the individuals retained a 'yellowish' tinted eye color, and no rays were visible extending from the caudal fin. Once eye color and caudal tail dimorphism were observed and noted, muscle and gonad tissue samples from selected individuals were processed for species identification and to determine their sex.

DNA barcoding analysis of samples from fish caught directly in the river and raised separately in tanks confirmed the presence of two species within the *Nematobrycon* genus. The emperor tetra (*N*. *palmeri*) is found exclusively in the Río San Juan basin, and the rainbow tetra (*N*. *lacortei*) is found in the Río Calima basin. Histology analysis also revealed that male emperor tetra have blue eyes, while males of rainbow tetra have red eyes. Males of both species also have a trident-shaped caudal fin. Females of both species have either 'pale gold' or 'metallic green' eyes, as previously described in the literature (Kyburz 1960; Axelrod 1971; Weitzman & Fink 1971; Aqualog 2016; Wikipedia 2025). Taken together, these observations confirm that juvenile males of emperor tetra and rainbow tetra can be practically distinguished within 2-3 months by eye color and a trident-shaped caudal fin (Figure 1). Females of both species have yellowish eyes and a lunate-shaped caudal fin, making them difficult to distinguish (Figure 1). In this study and by García (2024), these differences were also confirmed through gonadal histology.

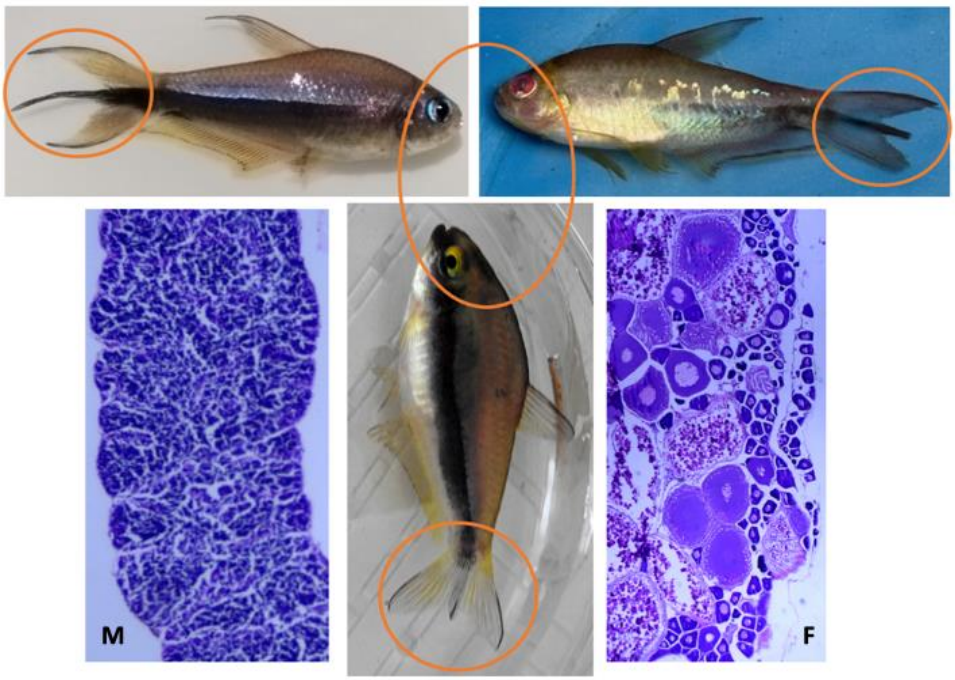

Figure 1. Practical differentiation between species and sexes of emperor and rainbow tetras. Male emperor tetras had blue eyes, while males of rainbow tetras had red eyes. Males of both species also had a trident-shaped caudal fin. Females of both species had the 'pale gold' or 'metallic green' eyes as previously described in the literature. Sexually dimorphic features verified with histology: M = male; F = female.

The locations where both species were caught were very close to each other and correspond to two new sight localities that slightly expand their narrow distribution to a more southerly range. The emperor tetra were caught in small creeks that flow into the lower Río San Juan between the towns of Palestina and Quícharo (Figure 2; 4°9′45.2″N, 77°8′8.0″W, Google coordinates). The rainbow tetra were collected in the lower Río

Calima (Figure 2; 4º09'37.3"N 77º08'38.0"W, Google coordinates). Previously, the species' geographical distributions were recorded only from the headwaters of the San Juan and Calima rivers (Weitzman & Fink 1971). The collection sites from this study extend the species ranges to the entire river drainage, and were not found reported in the scientific taxonomic databases such as FishBase, the Global Biodiversity Information Facility (GBIF), nor noted in the reviewed literature of popular aquarium magazines (websites), and scientific publications (Scopus and Web of Science).

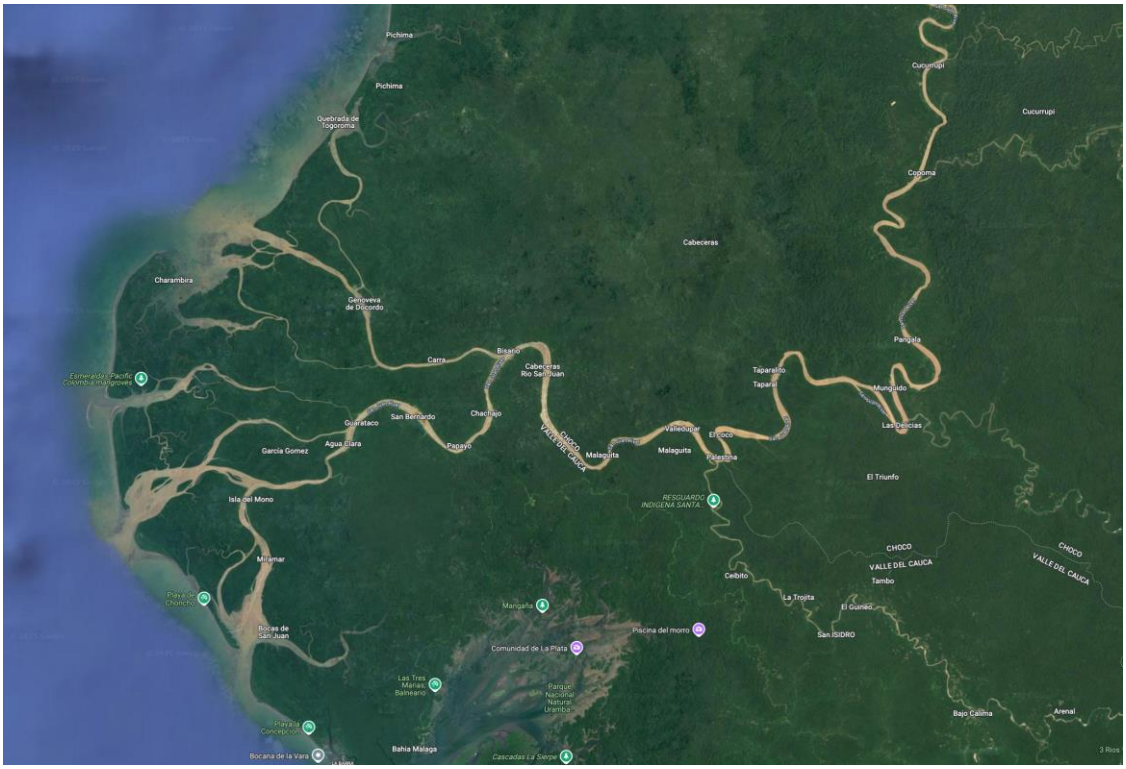

Figure 2. Collection areas of Emperor and Rainbow Tetras. These endemic species are found only in the San Juan and Calima river basins in tropical Colombia. FishBase (2025) and Google Maps (2025: 4.1625 N, 77.1356 W); use magnification to visualize localities better.

Species-specific range limits and accurate species identification are essential for species conservation, especially for understanding ecological niche adaptations and dispersal abilities (Sexton et al 2009). Unfortunately, the lack of such information often leads to confusion when dealing with ornamental fish collected from the wild. This confusion occurs when the fish species or biological sample is only available through a distribution channel with many intermediaries, rather than from its original source. As a result, much of the taxonomic and natural history information about the species lacks context, which can cause errors and taxonomic confusion. Clearly, this is reflected in the use of one common name (the 'emperor tetra') for two species of the *Nematobrycon* genus.

Conclusions. Most 'emperor tetras' available in the ornamental fish trade are two sister species, *Nematobrycon palmeri* and *N*. *lacortei*. Both are still wild-caught in remote fishing areas in tributaries flowing into the Río San Juan, located on Colombia's tropical Pacific coast. However, when caught, the fish are small juveniles and difficult to tell apart. They are primarily differentiated by the river basin where they were caught. This study showed that individuals of the two species can be differentiated at relatively small sizes (at 2.8±0.2 cm and 0.253±0.54 g). This research further confirms what aquarium enthusiasts have observed. Not only do our external observations of individual fish support these findings, but histological analysis showed that male emperor tetras have blue eyes, whereas male rainbow tetras have red eyes. Males of both species have a trident-shaped caudal fin. Females of both species display either 'pale gold' or 'metallic green' eyes. Additionally, DNA barcoding and site-specific fishing locations verify and

expand the known ranges of the emperor tetra in the Río San Juan river basin and of the rainbow tetra in the Río Calima, a major tributary of the Río San Juan.

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Conflict of interest. The authors declare that there is no conflict of interest.

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