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Unveiling the Secrets of South American Killifish: New Genera and their Evolution

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A recent study investigating the evolution of *Austrolebias* fish has yielded significant findings regarding their classification and evolutionary history. This study has led to the division of this group into 11 genera, including the identification of 4 new ones, providing valuable insights into the evolution and characteristics of these unique fish. Here, we highlight some of the key aspects of this research that will undoubtedly contribute to future studies and conservation strategies for these species and their habitats.

Studying the evolutionary relationships among organisms is a fundamental branch of biology, and since Darwin's time, it has been crucial in understanding how organisms have evolved, changed, and adapted over time. Zoological taxonomy, on the other hand, is a discipline that deals with naming and classifying living organisms into categories such as species, genera, families, and other taxonomic groups. Phylogenies, in turn, are tools that allow us to reconstruct the evolutionary history of groups, generating phylogenetic trees that reflect the patterns of life's diversification and are essential for generating natural classifications that reflect it.

In this article, we present a recent study on the phylogeny and taxonomy of a group of South American fish belonging to a species group popularly known as killifish. These fish inhabit temporary aquatic environments that dry up seasonally, and due to their restricted geographic distribution, many species are threatened to some degree. For this reason, it is important to study their diversity and evolution to develop effective conservation strategies and ecosystem management.

Our study, published in the scientific journal Zoological Journal of the Linnean Society, proposes a new phylogenetic hypothesis (a tree of kinship relationships among species) for the species formerly grouped in the genus *Austrolebias*. This phylogenetic analysis is the most comprehensive for this group to date and was based on the analysis of 10 different genes (6 nuclear and 4 mitochondrial) and 191 morphological characters, encompassing 90% of the species previously included in this genus. Additionally, it provides a redefinition and anatomical characterization of the former subgenera within the group that are elevated to genera. Four new genera are also established to accommodate the taxonomy of these species based on the generated evolutionary hypothesis. Lastly, a new species, *Argolebias guarani*, is described, which will be addressed in a separate article. In summary, our study contributes to the knowledge of the evolution and taxonomy of South American killifish, offering a new phylogenetic hypothesis and a better taxonomic organization for the group.

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Pictures: Pablo Calviño, Wilson S. Serra, Matheus V. Volcan, Marco Vaccari

Interesting fact.

Darwin and Wallace's work on evolution by natural selection was jointly presented at the meeting of the Linnean Society of London on July 1, 1858, and subsequently published in the Journal of the Proceedings of the Linnean Society in August of the same year (a precursor to the Zoological Journal of the Linnean Society where our article is published). The publication is titled "*On the Tendency of Species to form Varieties; and on the Perpetuation of Varieties and Species by Natural Means of Selection*" and consists of two articles: one written by Darwin and another by Wallace. In this publication, the authors present the theory of natural selection as the main mechanism of evolution and propose that species change gradually over time due to natural selection and hereditary variation.

Background

Several years ago, along with several colleagues, we decided to study the evolutionary relationships of the genus *Austrolebias*, a group of South American fish that inhabit ephemeral aquatic environments that completely dry up during part of the year. *Austrolebias* belong to the Rivulidae family, which is closely related to the African Nothobranchiidae family. Both families have diverse genera that may or may not exhibit a seasonal cycle. In both cases, species from these families are highly valued in the aquarium trade due to their attractive colors, striking behavior, and unique life cycle. These fish survive as embryos in eggs buried in the mud, which the parents deposit while the pool has water. Towards the end of the season, the adults die due to desiccation. With the arrival of the rains, the pools fill up, the eggs hatch, and the cycle restarts.

These "rockers" fish live fast and die young. They have a very accelerated metabolism, grow at an exceptionally fast rate, and reach sexual maturity in a matter of weeks or months, as they generally only live for a few months before the pool completely dries up. Within this group, we find the fish of the *Austrolebias* genus particularly interesting. They inhabit our region (central and northern Argentina, southeastern Bolivia, Paraguay, Uruguay, and southern Brazil) in the La Plata Basin and surrounding areas. This genus comprises more than 50 species, many of which are endangered primarily due to habitat loss. Some of the most noticeable characteristics that distinguish them from other rivulid genera at first sight are the presence of a dark vertical band below the eye, unpair fins with rounded ends, and a preopercular region without scales.

In 1998, Brazilian researcher Wilson Costa established the genera *Austrolebias* and *Megalebias* after conducting a phylogenetic analysis that encompassed a large portion of South American annual killifish. Later, in 2006, the same author published a taxonomic revision focused on *Austrolebias* and considered *Megalebias* as a synonym of the previous genus, as the species in that group were nested among *Austrolebias* species in his phylogeny. In that work, he described several new species and conducted a phylogenetic analysis based on morphological information to establish several species groups. A few years later, he published the book "Catalog of Aplocheiloid Killifishes of the World," in which he created subgenera within the *Austrolebias* genus based on the species groups he had identified in his previous analysis: *Acantholebias*, *Acrolebias*, *Argolebias*, *Austrolebias*, *Cypholebias*, *Gymnolebias*, and *Megalebias*.

Parallel to this, Uruguayan researcher Graciela García, along with several collaborators, published a series of papers (e.g., in 2000, 2002, and 2014) in which the genus *Austrolebias* was analyzed based on genetic information instead of morphology. These analyses yielded some species groupings similar to those obtained by Costa in his morphological studies, but they differed mainly in the relationship between these groups and the affinity of certain species, such as *Argolebias nigripinnis*, *Amatolebias patriciae*, or *Megalebias wolterstorffi*, which appeared in different parts of each phylogeny.

In 2018, two independent studies were published that took a combined approach, analyzing both genetic and morphological information in the same analysis. The study led by Argentine researcher Felipe Alonso, along with several collaborators, took the morphological information (83 characters) published by Costa in 2006 and combined it with a large portion of the genetic information (Cytochrome-b gene) generated by García. They also incorporated some species not included in previous analyses, such as *Austrolebias wichi*, which was described as a new species in the same work. On the other hand, the study led by Uruguayan researcher Marcelo Loureiro presented an even broader dataset, combining 173 morphological characters with sequences from four different genes. Similarly to previous publications, both studies obtained several groupings of similar species but with different evolutionary relationships between the groups. The results from Loureiro and colleagues were more robust due to the larger amount of information. In this latter study, the authors obtained partially coincident groupings with the subgenera proposed by Costa in 2008, but many species fell into two groups without a subgenus.

In our recent study (Alonso *et al.*, 2023), we conducted a new phylogenetic analysis of the *Austrolebias* genus and proposed a new taxonomic arrangement for this group of species, which includes dividing the genus into 11 different genera, elevating the 7 pre-existing subgenera to the genus level, and creating 4 new genera (Table 1). Additionally, we described a new species, *Argolebias guarani*, which will be discussed separately. This new phylogenetic hypothesis was obtained through the analysis of 10 different genes and 191 anatomical characters, including 90% of the valid species in the group (47 out of 54). In the following sections, we will share some of the results from this study and the definitions of the proposed new genera.

Austrolebias genera group

Based on the obtained results, we have divided the genus *Austrolebias* into 11 different genera. Seven of these genera correspond to the subgenera proposed by Wilson Costa, which we have elevated to the genus level, although the

Table 1. Main changes in the generic names of the Austrolebias genus group. *New taxa created in the respective studies.

<table>
<tr><th>Costa (1998) splits Cynolebias into 3 genera</th><th>Costa (2006) synonimizes Megalebias</th><th>Costa (2008), subgenera of Austrolebias</th><th>Loureiro et al. (2018)</th><th>In this study, genera</th></tr>
<tr><td>Cynolebias Steindachner 1876</td><td>Cynolebias</td><td>Cynolebias</td><td>Cynolebias</td><td>Cynolebias</td></tr>
<tr><td rowspan="9">*Austrolebias Costa 1998</td><td rowspan="11">Austrolebias</td><td rowspan="3">subg. Austrolebias</td><td>"unnamed subgenus"</td><td>*Amatolebias</td></tr>
<tr><td>subg. Austrolebias</td><td>Austrolebias</td></tr>
<tr><td rowspan="2">subg. Acrolebias</td><td>*Garcialebias</td></tr>
<tr><td>subg. *Acrolebias Costa 2008</td><td>Acrolebias</td></tr>
<tr><td>subg. *Acantholebias Costa 2008</td><td>subg. Acantholebias</td><td>Acantholebias</td></tr>
<tr><td>subg. *Gymnolebias Costa 2008</td><td>subg. Gymnolebias</td><td>Gymnolebias</td></tr>
<tr><td>subg. *Cypholebias Costa 2008</td><td>subg. Cypholebias</td><td>Cypholebias</td></tr>
<tr><td rowspan="2">subg. *Argolebias Costa 2008</td><td>" unnamed subgenus "</td><td>*Matilebias</td></tr>
<tr><td>subg. Argolebias</td><td>Argolebias</td></tr>
<tr><td rowspan="2">*Megalebias Costa 1998</td><td rowspan="2">subg. Megalebias (synonym of Austrolebias, valid as subgen.)</td><td rowspan="2">subg. Megalebias</td><td>Megalebias</td></tr>
<tr><td>*Titanolebias</td></tr>
</table>

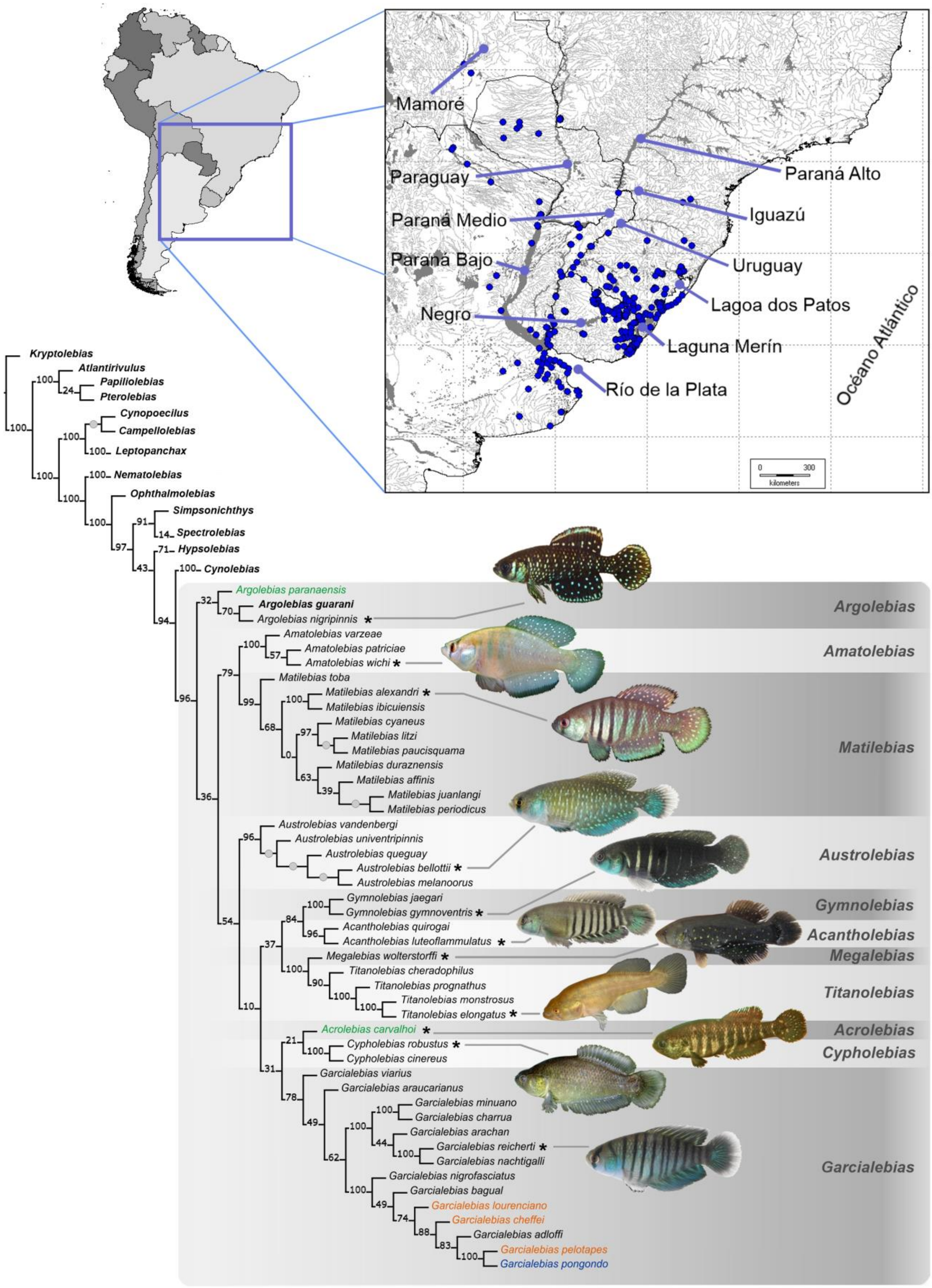

Figure 1. Top. Distribution of species in the "*Austrolebias* genus group" and major basins in southern South America. Bottom. Phylogeny based on morphology and genetics of the "*Austrolebias* genus group" (modified from Alonso *et al.*, 2023).

species composition in some cases has changed slightly. Additionally, we have created four new genera (Figure 1). Together, they form what we call the Group of *Austrolebias* Genera, which is closely related to the genus *Cynolebias*. These genera are: *Acantholebias* Costa, 2008, *Acrolebias* Costa, 2008, *Amatolebias* Alonso *et al.*, 2023, *Argolebias* Costa, 2008, Austrolebias Costa, 1998, *Cypholebias* Costa, 2008, *Garcialebias* Alonso *et al*., 2023, *Gymnolebias* Costa, 2008, *Matilebias* Alonso *et al*., 2023, *Megalebias* Costa, 1998, and *Titanolebias* Alonso *et al*., 2023.

The members of this group exhibit unique characteristics, known as diagnostic characters, which collectively distinguish them from other genera within the family. These include certain bone and morphological traits, the absence of scales in the head region below the eye, and the presence of a dark vertical band below the eye.

***Argolebias* Costa, 2008 (Figure 2)**

Type species: *Cynolebias nigripinnis* Regan, 1912

Etymology: "*argos*" = bright, "*lebias*" = small fish.

It was originally proposed as a subgenus within *Austrolebias*, to group species with "dark gray pectoral fins with blue iridescence in males," including *Matilebias affinis*, *M. alexandri, M. ibicuiensis, M. periodicus, M. litzi, M. cyaneus, M. juanlangi, M. toba, A. paranaensis*, and *A. nigripinnis*. Subsequently, Loureiro and colleagues (2018) restricted the use of the subgenus *Argolebias* only to *A. nigripinnis*. Regarding *A. paranaensis*, no decision was made as it was not included in the analyses. In our work, we elevate *Argolebias* to the genus level, encompassing the following species: *Argolebias guarani, Argolebias nigripinnis* and *Argolebias paranaensis*.

The genus *Argolebias* can be recognized by the following unique combination of characteristics: 1) presence of iridescent spots on the middle region and margin of the pectoral fins in males, 2) wide dark gray to black margin on the flank scales below the lateral line and above the anal fin, 3) fused iridescent spots on the margin of the dorsal fin in

Figure 2. *Argolebias.* *Argolebias guarani* (A-B), Misiones, Argentina. *Argolebias nigripinnis,* Colonia, Uruguay (C), and Buenos Aires, Argentina (D). Photographs: Pablo Calviño (A, B, D) and Sebastián Serra (C).

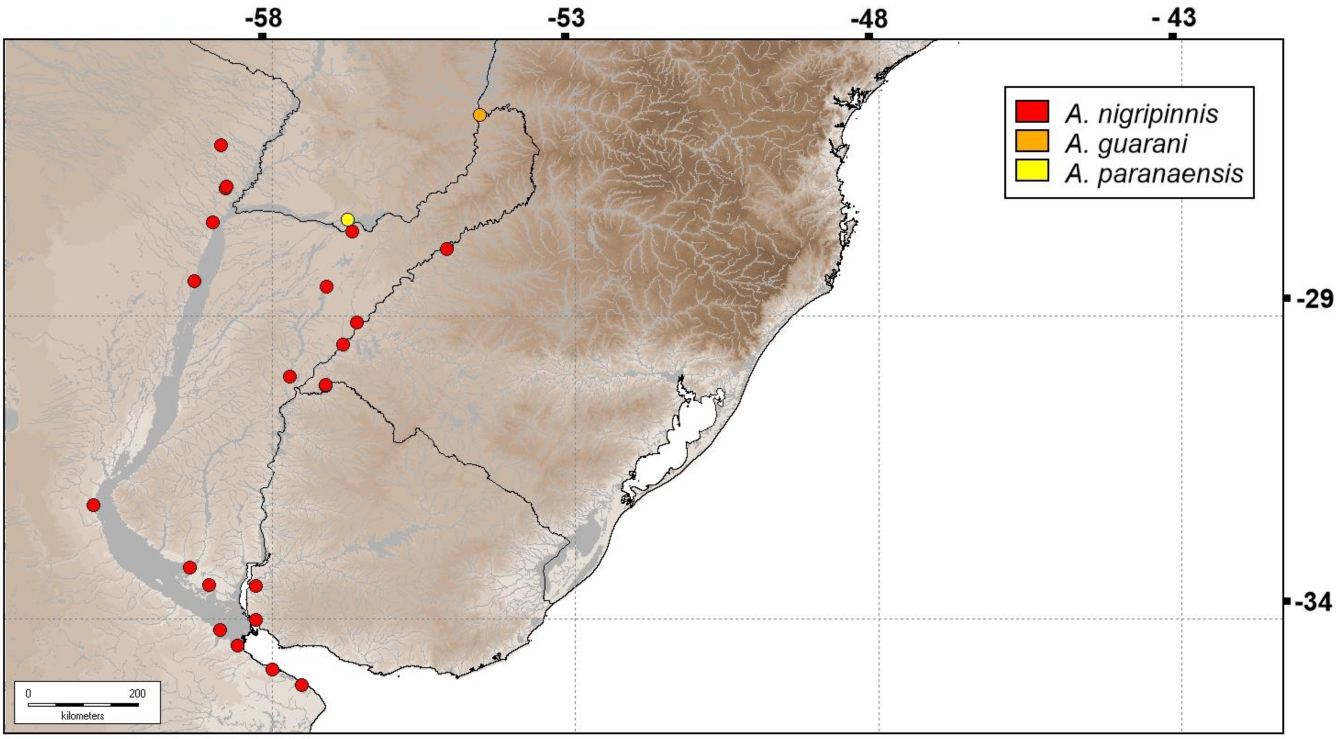

Figure 3. Known geographic distribution of species in the genus *Argolebias*.

adult males, 4) presence of a dark spot at the start of the dorsal fin, and other anatomical characters.

Argolebias paranaensis is assigned to this genus based on anatomical characters of preserved specimens, as it is only known from the original specimens collected over 40 years ago in Ayolas, southern Paraguay. The coloration in life of this species is unknown, and no DNA sequences are available.

The distribution of the genus *Argolebias* encompasses the floodplains associated with the middle and lower Uruguay River, lower Paraguay River, middle and lower Paraná River, and Río de la Plata, in Argentina, Uruguay, Brazil, and Paraguay (Figure 3).

***Amatolebias* Alonso *et al*., 2023 (Figure 4)**

Type species: *Austrolebias wichi* Alonso, Terán, Calviño, García, Cardoso & García, 2018

Etymology: dedicated to the Uruguayan naturalist Luis H. Amato, "*lebias*" = small fish.

In this genus, we group species that have been linked to different groups and subgenera over time, such as *Acrolebias*, "*Austrolebias alexandri* species group" and "*Austrolebias bellottii* group". Specifically, it is formed by the following species: *Amatolebias patriciae*, *Amatolebias varzeae* and *Amatolebias wichi*.

The genus *Amatolebias* can be recognized by the following characteristics: 1) males with dark vertical bars only in the anterior region of the body, 2) scales on the flanks between the anal and dorsal fins with a bluish to greenish leaden color, without dark borders, 3) anal fin with a black margin, 4) scales below the dorsal fin with a thin light gray to gray-brown border, and other morphological characters.

The genus *Amatolebias* is distributed in the Chaco region of Argentina and Paraguay, as well as in the upper Uruguay River basin in Brazil (Figure 6).

Figure 4. *Amatolebias.* *Amatolebias wichi* (A-B), Salta, Argentina. *Amatolebias patriciae* (C-D), Chaco, Argentina. *Amatolebias varzeae* (E-F), Rio Grande do Sul, Brasil. Photographs: Pablo Calviño (A, B, C, D) and Matheus Volcan (E, F).

***Matilebias* Alonso *et al.*, 2023 (Figure 5)**

Type species: *Cynolebias alexandri* Castello & López, 1974

Etymology: dedicated to the Argentine researcher Matías Pandolfi, "*lebias*" = small fish.

In this genus, we group many of the species that were originally included in *Argolebias* or mentioned as belonging to the "*alexandri* group": *Matilebias affinis, Matilebias alexandri, Matilebias camaquensis, Matilebias cyaneus, Matilebias duraznensis, Matilebias ibicuiensis, Matilebias juanlangi, Matilebias litzi, Matilebias paucisquama, Matilebias periodicus* and *Matilebias toba*.

The genus is characterized, among other things, by the following distinctive features in males: 1) presence of vertically aligned iridescent spots throughout the flank, forming lines or bands, 2) border of scales below the dorsal fin with a light gray to gray-brown color, and 3) absence of iridescent spots in the middle and distal region of the pectoral fins (unlike what is observed in *Argolebias*). In addition, females have one or more irregularly arranged black spots in the posterior region of the flank and caudal peduncle.

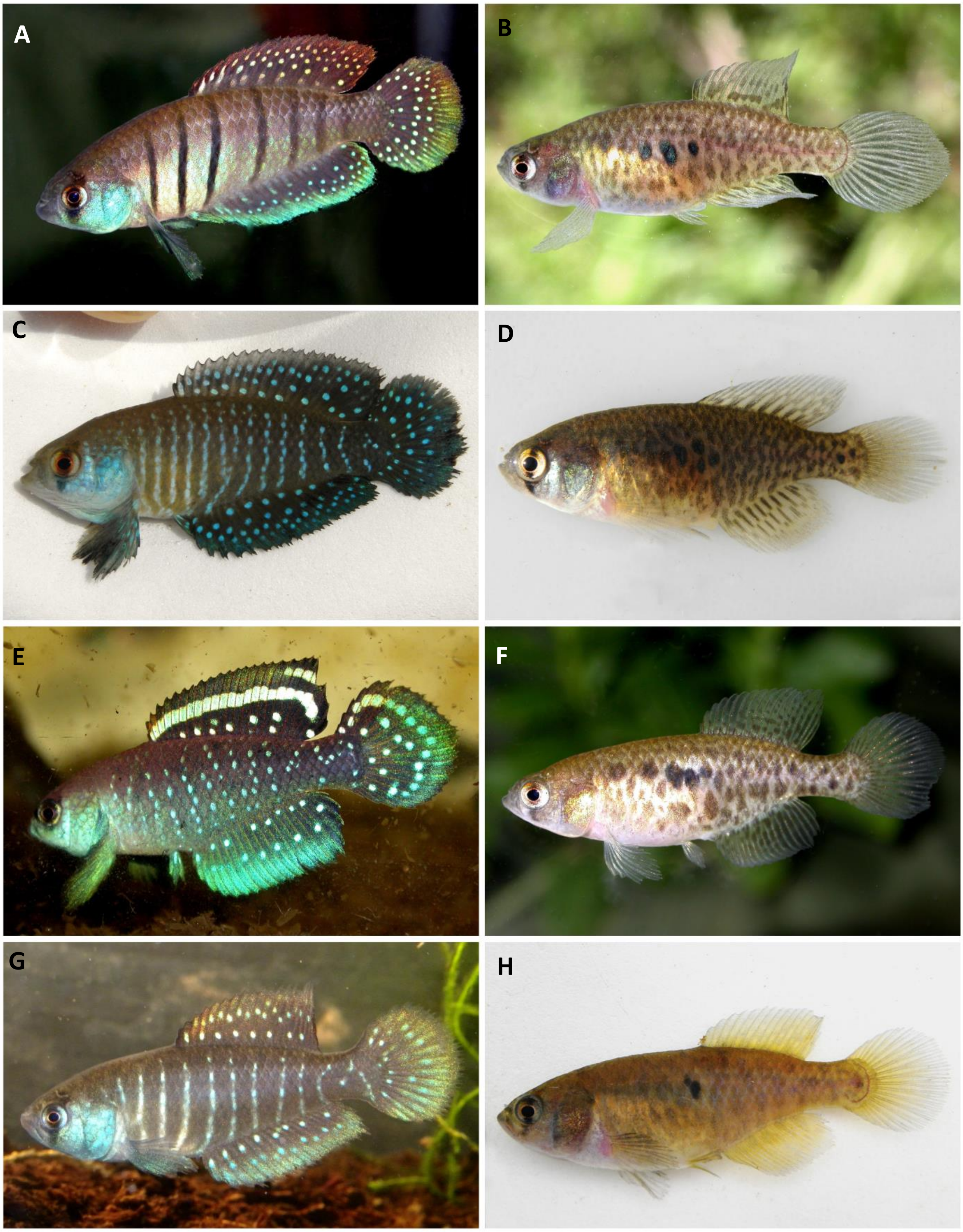

Figure 5. ***Matilebias***. *Matilebias alexandri* (A-B), Entre Ríos, Argentina. *Matilebias periodicus* (C-D), Rio Grande do Sul, Brasil. *Matilebias toba* (E-F), Chaco, Argentina. *Matilebias juanlangi* (G-H), Cerro Largo, Uruguay. Photographs: Pablo Calviño (A, B, E, F) and Sebastián Serra (C, D, G, H).

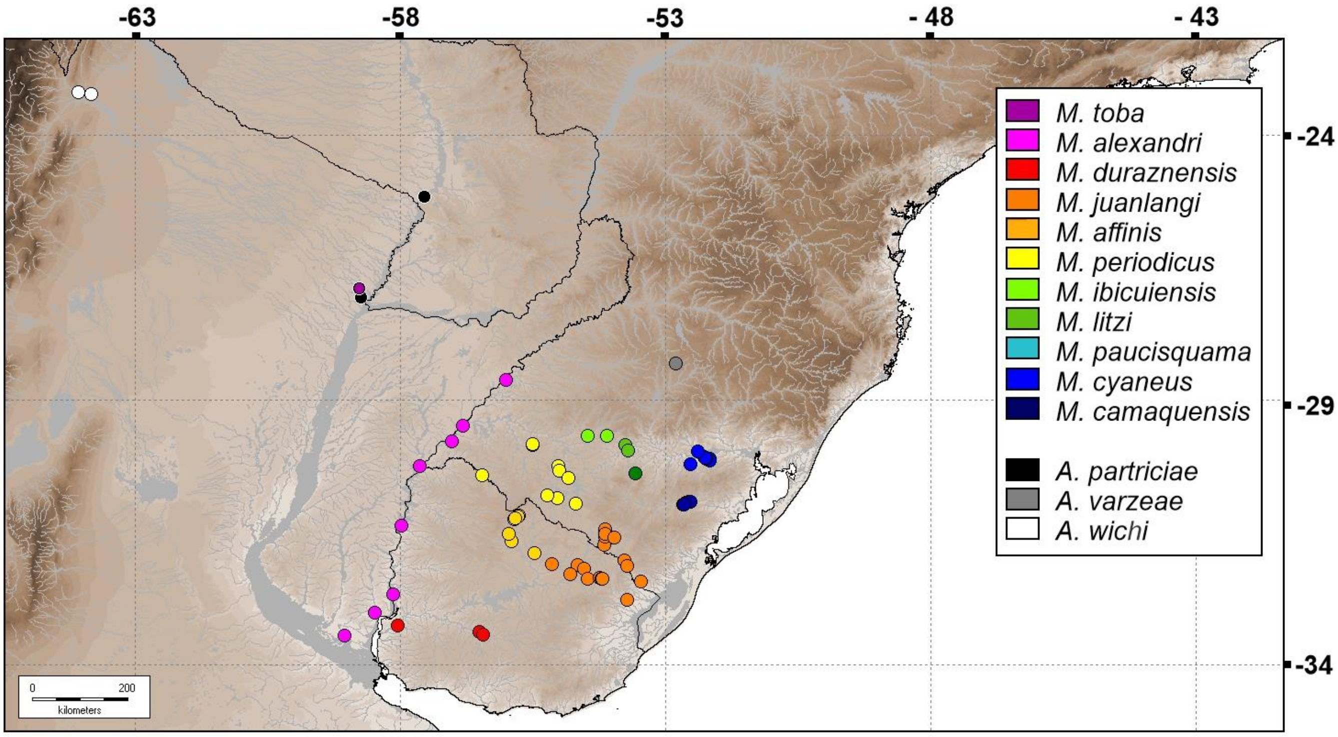

Figure 6. Reported geographic distribution of species from the genera *Matilebias* and *Amatolebias*.

The genus *Matilebias* is distributed in the lower basins of the Paraguay and Paraná rivers, as well as in the middle and lower basin of the Uruguay River and in the Patos-Merín lagoon system, in Argentina, Brazil, and Uruguay (Figure 6). Although there are informal reports on social media about the possible presence of the species *M. toba* in Paraguay, its existence in this country still needs to be confirmed.

***Austrolebias* Costa, 1998 (Figure 7)**

Type species: *Cynolebias bellottii* Steindachner, 1881

Etymology: "*australis*" = southern, "*lebias*" = small fish.

The genus *Austrolebias* was proposed in 1998 to group a set of species that were previously classified under the genus *Cynolebias*. In 2006, Costa synonymized *Megalebias* with *Austrolebias*, as his phylogenetic analysis revealed that the species of the former grouped nested within the *Austrolebias* species. Originally, the subgenus *Austrolebias* was used to group the species of the "*bellottii* group," which now form the genus *Austrolebias* in the strict sense, as well as the species of the "*adloffi* group" (now in *Garcialebias*) and *Amatolebias patriciae*. However, Loureiro and collaborators restricted the use of the subgenus *Austrolebias* solely to the species of the "*bellottii* group."

In our study, we restrict this genus to the following species: *Austrolebias accorsii, Austrolebias bellottii, Austrolebias ephemerus, Austrolebias melanoorus, Austrolebias queguay, Austrolebias univentripinnis* and *Austrolebias vandenbergi*.

The distinctive characteristics of this genus include: 1) the triangular shape of the anal fin in females, with the anterior rays proportionally longer, 2) the pectoral fins of males being hyaline with a black margin and an iridescent bluish-green submargin, except in *A. melanoorus*, which lacks the iridescent submargin, and 3) non-aligned dark spots on the caudal peduncle of females.

The distribution of the genus encompasses a large part of the Río de la Plata basin (excluding the middle and upper regions of the Paraná and

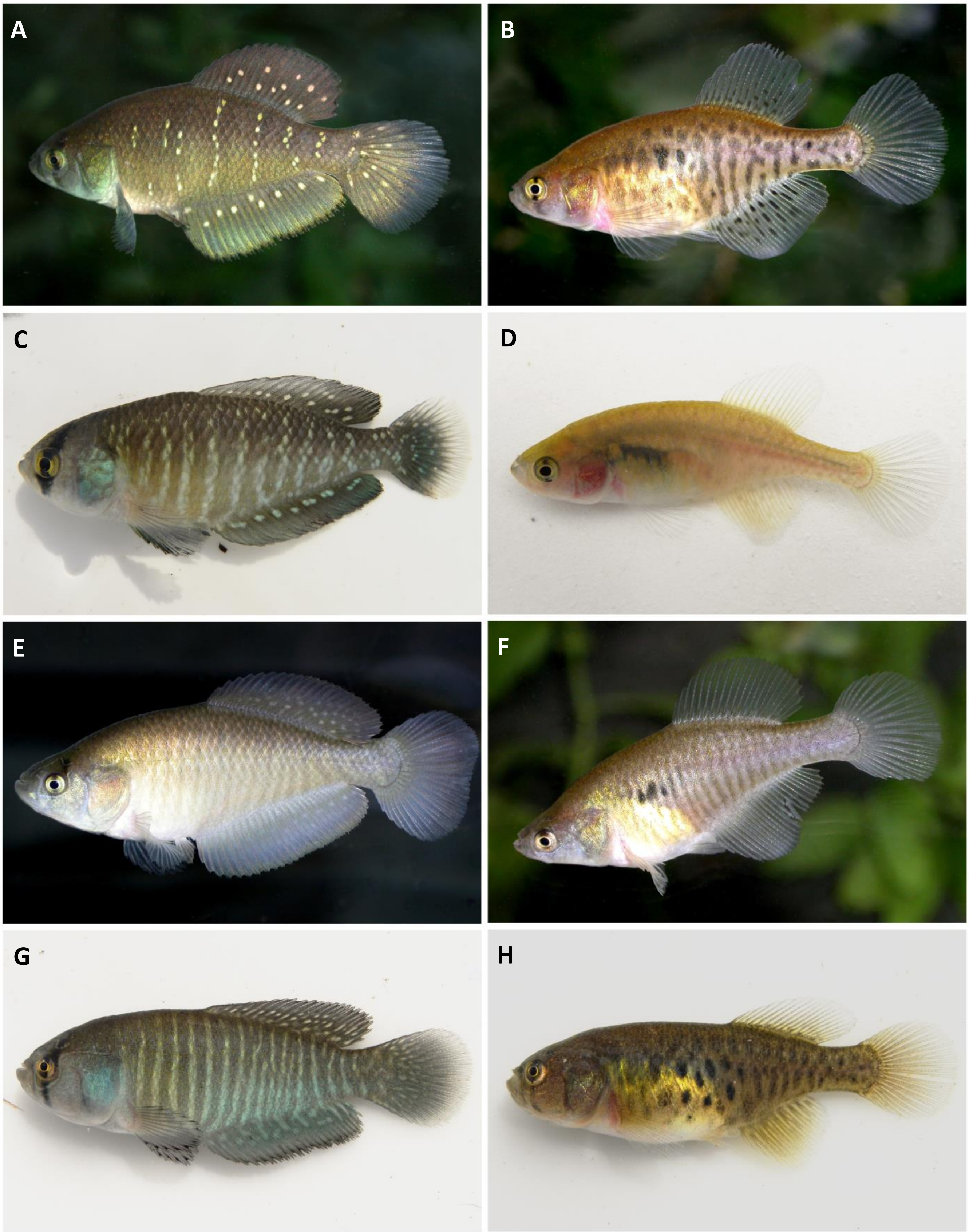

Figure 7. ***Austrolebias.*** *Austrolebias bellottii* (A-B), Buenos Aires, Argentina. *Austrolebias univentripinnis* (C-D), Cerro Largo, Uruguay. *Austrolebias vandenbergi* (E-F), Salta, Argentina. *Austrolebias melanoorus* (G-H), Tacuarembó, Uruguay. Photographs: Pablo Calviño (A, B, E, F) and Sebastián Serra (C, D, G, H).

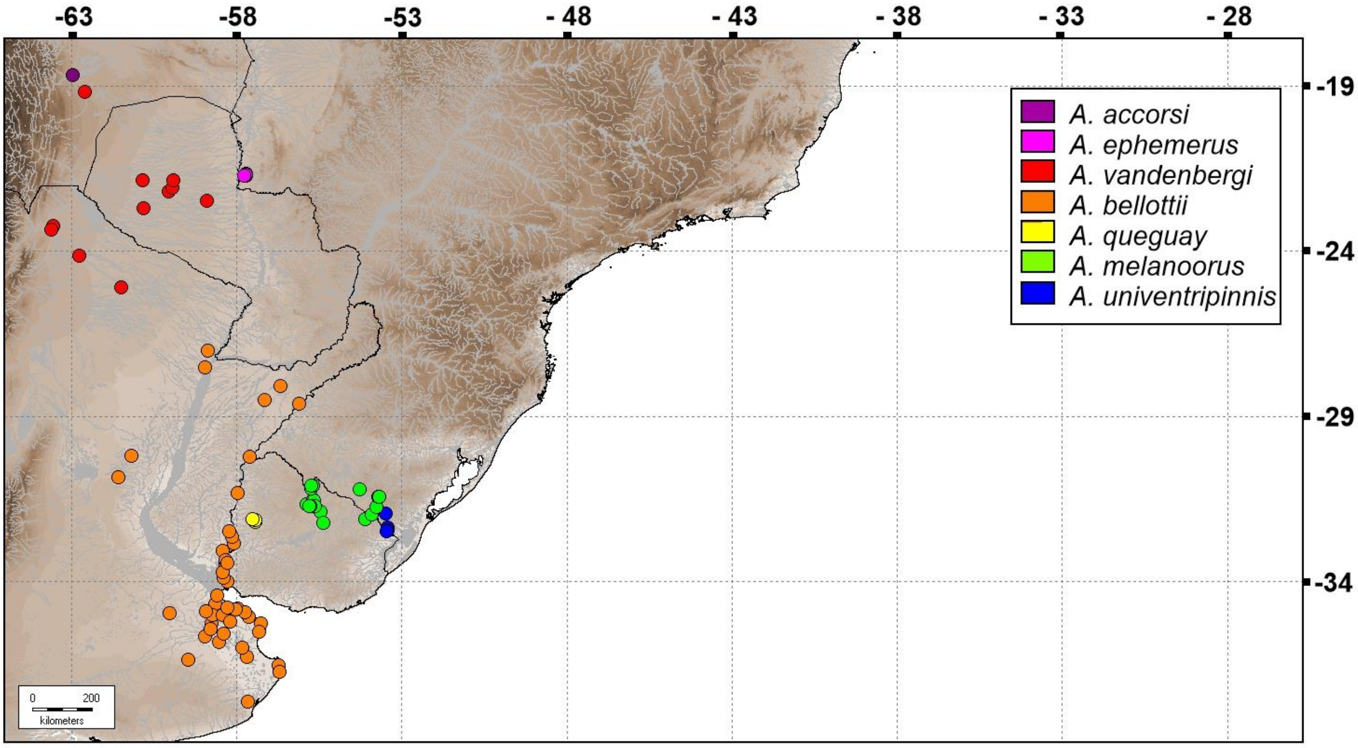

Figure 8. Reported geographic distribution for the species of the genus *Austrolebias*.

Uruguay basins), the Laguna Merín basin, and the headwaters of the Mamoré River basin, in Argentina, Uruguay, Brazil, Bolivia, and Paraguay (Figure 8).

***Gymnolebias* Costa, 2008 (Figure 9)**

Type species: *Cynolebias gymnoventris* Amato, 1986

Etymology: "*gymnos*" = naked (referring to the ventral region without scales), "*lebias*" = small fish.

The genus *Gymnolebias* was originally proposed as a subgenus of *Austrolebias* in 1998 to group species with the following characteristics: "ventral region without scales, sub and supraorbital regions without dark marks, and dark brown to black flanks in males without bright marks and with light gray bands". Both in its original use as a subgenus and in its current use as a genus, the composition of species in this group has remained the same and has been consistent in all phylogenetic analyses based on morphological and genetic data that have been conducted over time. Specifically, *Gymnolebias* is composed of the following species: *Gymnolebias gymnoventris* and *Gymnolebias jaegari*.

In its current definition, the genus *Gymnolebias* is characterized by: 1) the absence of scales in the ventral region of the body, 2) the presence of inconspicuous or absent dark bands below and above the eye, and 3) the dark brown to black flanks in males with vertical light gray bands, which are wider in the anterior region.

So far, the presence of *Gymnolebias* has been recorded in the basins associated with the Patos-Merín system of lagoons in Brazil and Uruguay (Figure 11).

***Acantholebias* Costa, 2008 (Figure 10)**

Type species: *Cynolebias luteoflammulatus* Vaz-Ferreira, Sierra de Soriano & Scaglia de Paulete, 1964

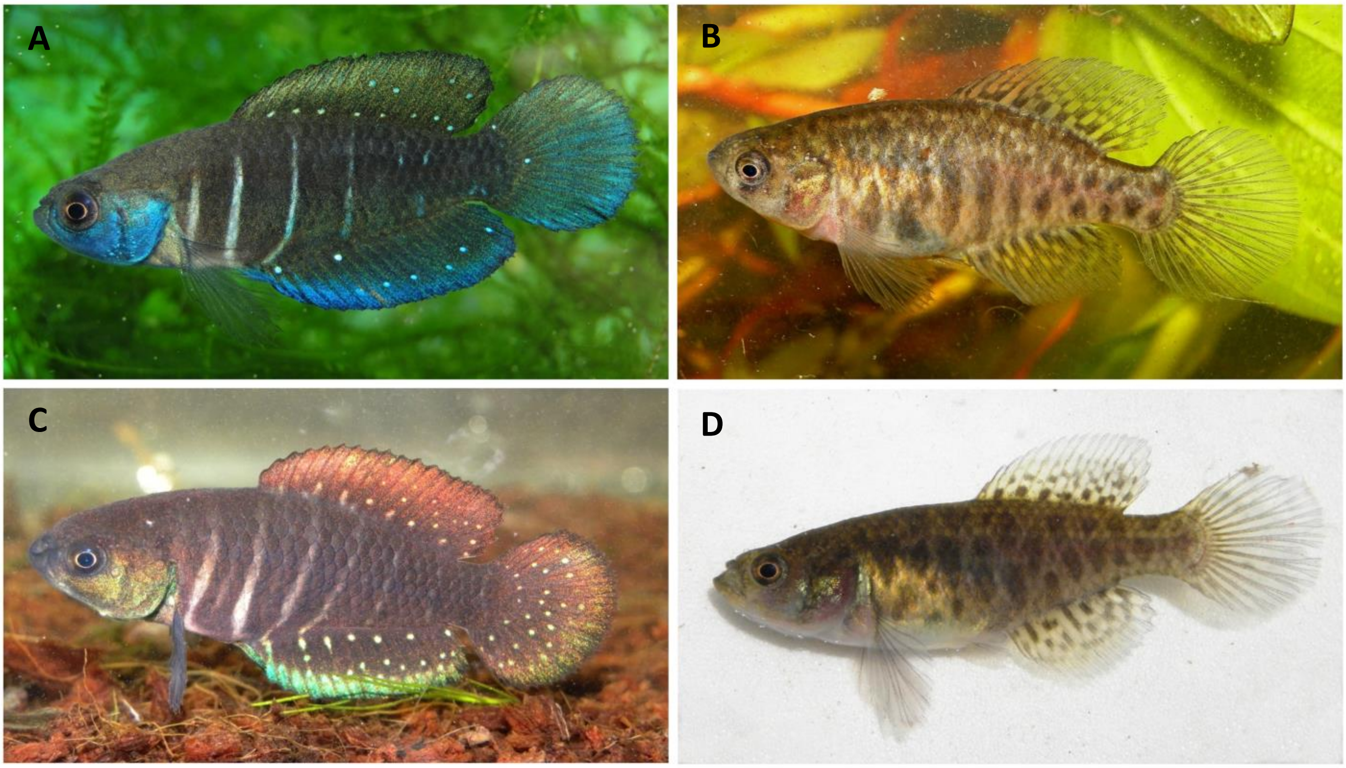

Figure 9. ***Gymnolebias.*** *Gymnolebias jaegari* (A-B), Rio Grande do Sul, Brasil. *Gymnolebias gymnoventris* (C-D), Lavalleja, Uruguay. Photographs: Matheus Volcan (A, B) y Sebastián Serra (C, D).

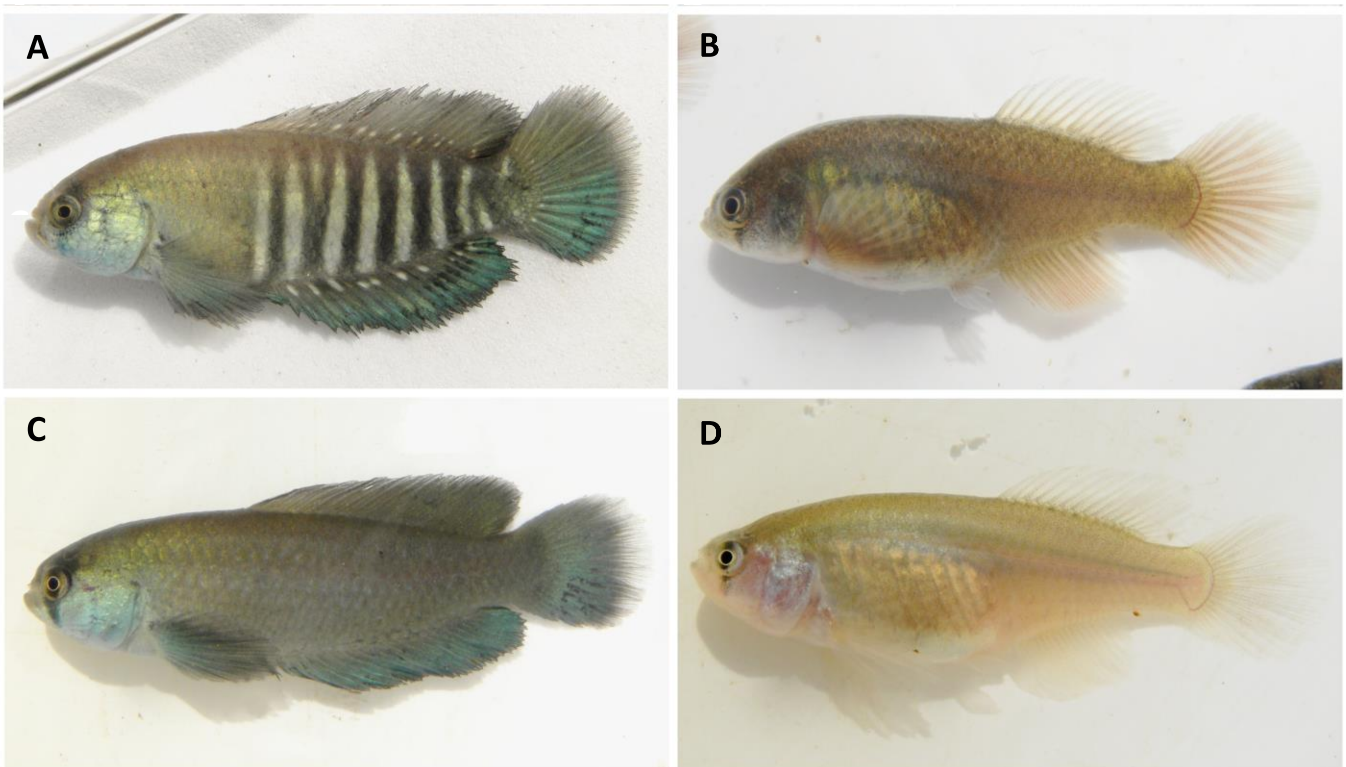

Figure 10. ***Acantholebias.*** *Acantholebias luteoflammulatus* (A-B), Rocha, Uruguay. *Acantholebias quirogai* (C-D), Cerro Largo, Uruguay. Photographs: Sebastián Serra.

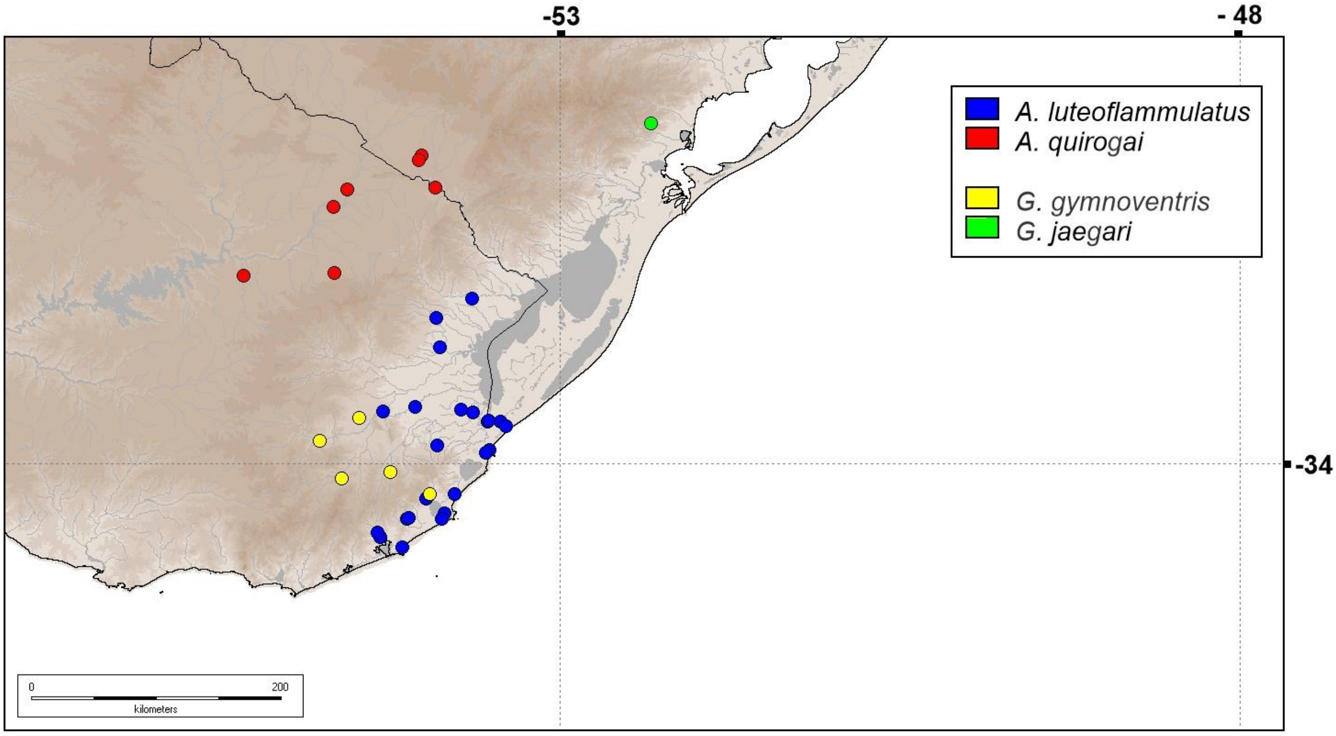

Figure 11. Reported geographic distribution for the species of the genera *Gymnolebias* and *Acantholebias*.

Etymology: "*akantha*" = spine (referring to the contact organs on the flanks and fins), "*lebias*" = small fish.

Acantholebias was originally created as a subgenus of *Austrolebias* in 1998 to include only *Acantholebias luteoflammulatus*. In 2018, Loureiro and colleagues described *Acantholebias quirogai* and included it as the second member of this subgenus. In our current work, we maintain the species composition of the genus with these two species: *Acantholebias luteoflammulatus* and *Acantholebias quirogai*.

This genus is characterized, among other features, by the following traits in adult males: 1) a wide border of scales on the dorsum ranging from light gray to gray-brown, 2) absence of iridescent marks on the flanks, 3) a dark vertical bar on the posterior part of the dorsal fin, which is more visible in juvenile individuals, and 4) bright bluish-green pectoral fins.

Acantholebias is distributed in the upper basin of the Rio Negro (lower Rio Uruguay), as well as in the eastern Atlantic basins of Uruguay and the basins associated with Laguna Merín, present in Brazil and Uruguay (Figure 11).

Megalebias Costa, 1998 (Figure 12)

Type species: *Cynolebias wolterstorffi* Ahl, 1924

Etymology: "*mega*" = large, "*lebias*" = small fish

Based on a phylogenetic analysis using morphological characters, Costa created the genus *Megalebias* in 1998 to group a set of large species that were previously classified under the genus *Cynolebias*, many of which were believed to be piscivorous. In a subsequent analysis in 2006, Costa found that these species formed a group nested within the genus *Austrolebias*. To avoid having *Austrolebias* as a "non-natural" group that included a common ancestor but not all its descendants, Costa decided to synonymize *Megalebias* with *Austrolebias*, making the latter valid, although he mentioned *Megalebias* as a valid subgenus without providing further details. This subgenus is confirmed in his 2008 work, where he proposed the other subgenera within *Austrolebias*.

In subsequent studies based on morphological data, it has been found that a group of species forms a cohesive group that includes *T. elongatus, T. monstrosus, T. cheradophilus, T. prognathus*, and *M. wolterstorffi*. However, most phylogenetic analyses based solely on molecular data (genes) find that *M. wolterstorffi* is not closely related to the other mentioned species. In our recent work, we obtained similar results. When combining morphology and gene data, we found *M. wolterstorffi* to be the sister species to the other species in the group, while when analyzing only molecular data, this species is placed distantly from this group within the tree. The apparent inconsistency between different analyses may be due to evolutionary convergence at the morphological level in large predatory or piscivorous species like *M. wolterstorffi* and the other species, leading to the independent evolution of many similar characteristics of species from different lineages. Therefore, the remaining species in this group are included in a new genus called *Titanolebias*.

Figure 12. ***Megalebias.*** *Megalebias wolterstorffi*, Rio Grande do Sul, Brasil. Photographs: Matheus Volcan.

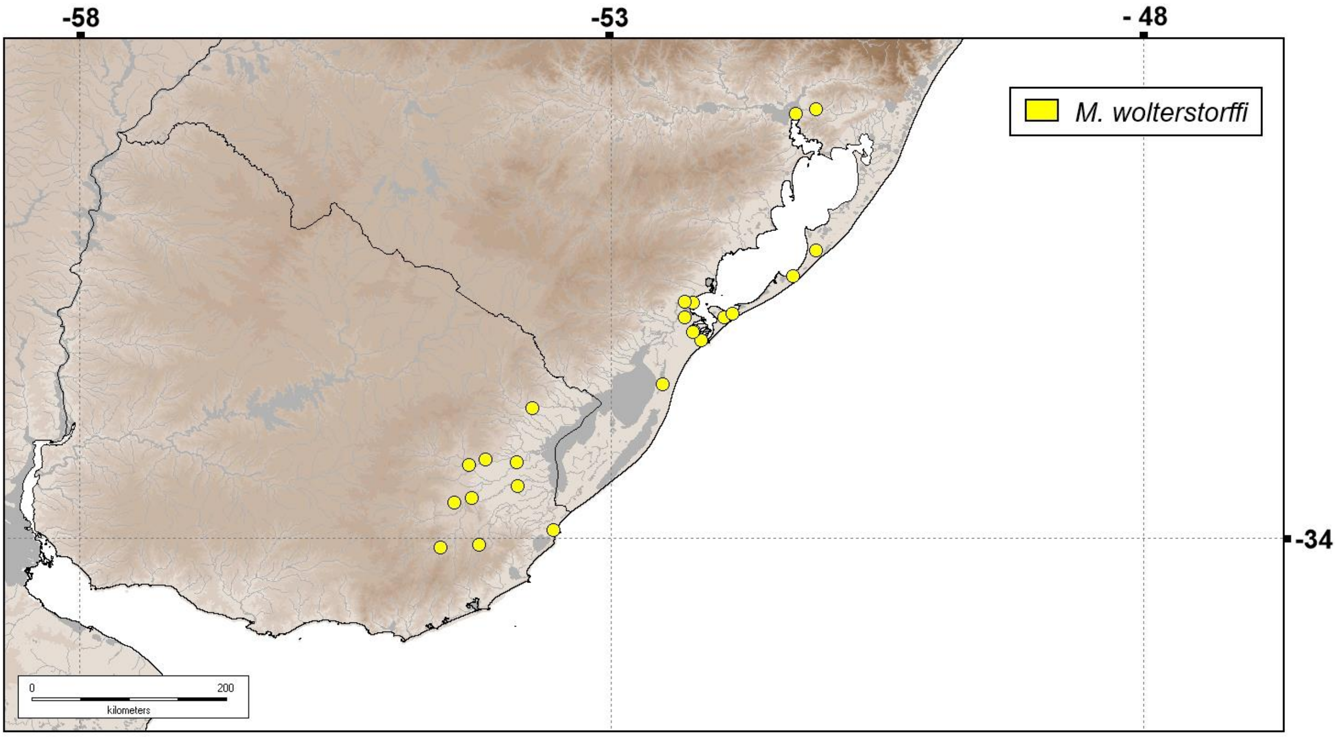

Figure 13. Reported geographic distribution for the species of the genus *Megalebias*.

As a result, the genus *Megalebias* now consists of a single species, *M*. *wolterstorffi*, which can be distinguished from other genera by a series of characteristics, such as the presence of 25 to 40 scales in the lateral line, a backward-directed suborbital bar, and the separation of the pelvic fins.

This species inhabits the floodplain and wetland areas of the Patos-Merín lagoon system in Uruguay and Brazil (Figure 13).

Titanolebias Alonso *et al*., 2023 (Figure 14)

Type species: *Cynolebias elongatus* Steindachner, 1881

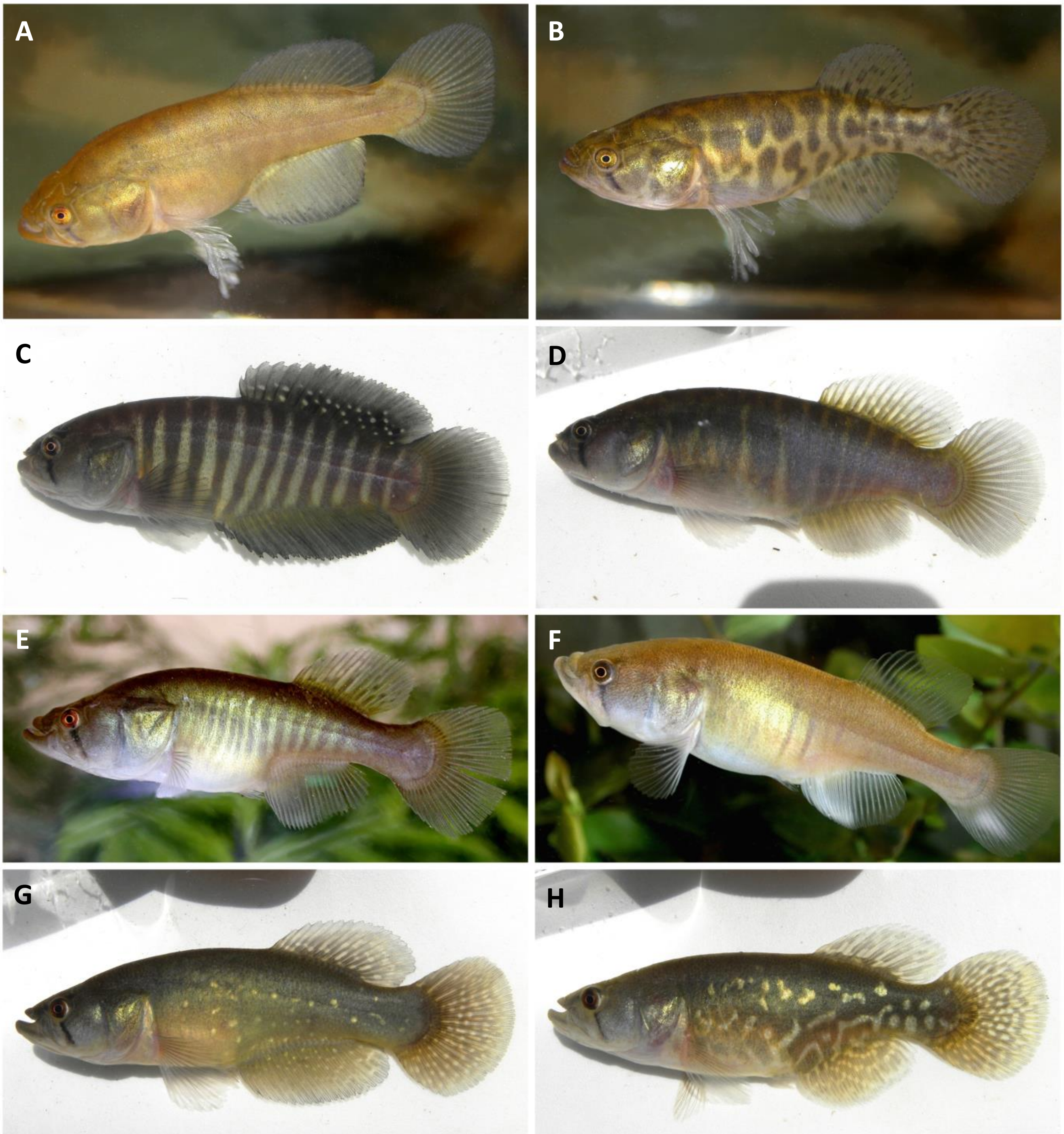

Figure 14. ***Titanolebias***. *Titanolebias elongatus* (C-D), Buenos Aires, Argentina. *Titanolebias cheradophilus* (E-F), Lavalleja, Uruguay. *Titanolebias monstrosus* (G-H), Salta, Argentina. *Titanolebias prognathus* (I-J) Lavalleja, Uruguay. Photographs: Pablo Calviño (A, B, E, F) and Sebastián Serra (C, D, G, H).

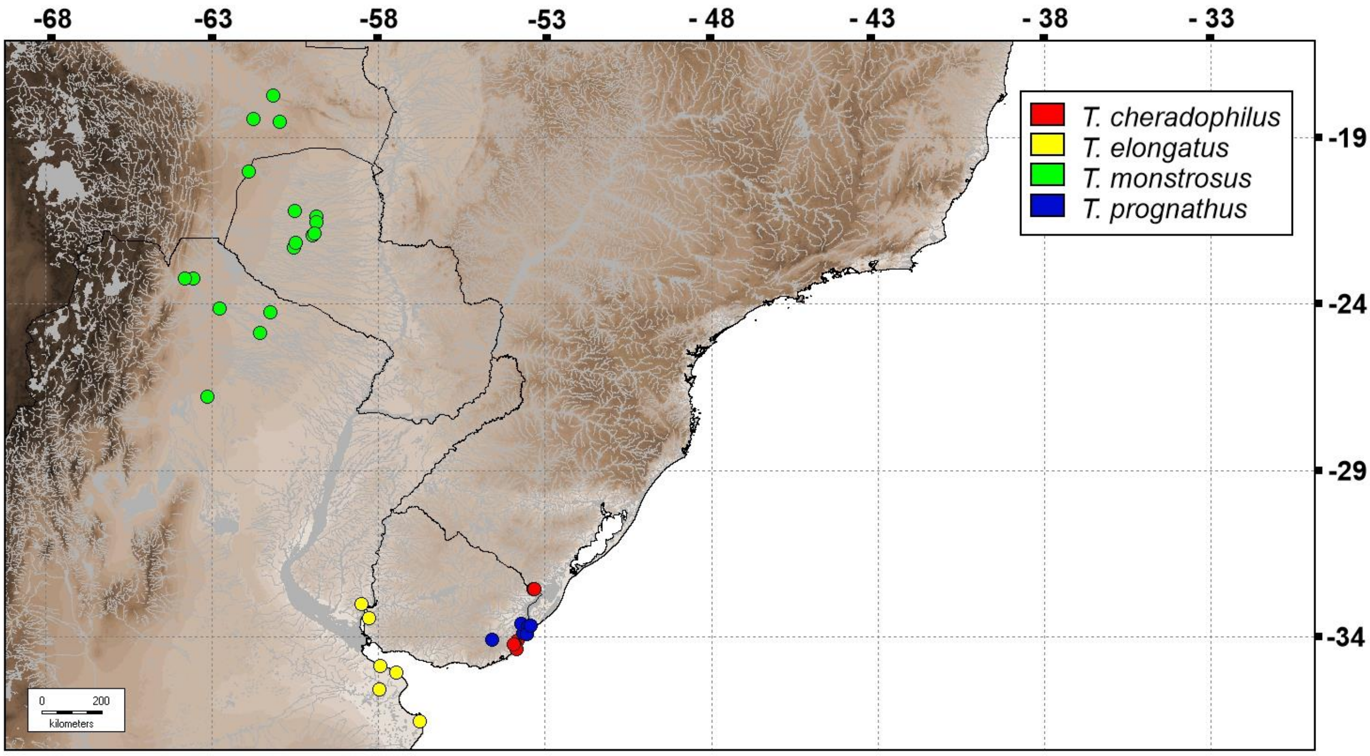

Figure 15. Reported geographic distribution for the species of the genus *Titanolebias*.

Etymology: From Greek mythology, the Titans (Τιτᾶνες, Titânes, singular: Τιτάν, -ήν, Titán) were pre-Olympian gods, children of the gods Uranus (Sky) and Gaia (Earth), known for their large size; "*lebias*" = small fish.

In *Titanolebias*, we have grouped species that were previously associated with the genus/subgenus *Megalebias*, except for *Megalebias wolterstorffi*. These species include *Titanolebias cheradophilus*, *Titanolebias elongatus, Titanolebias monstrosus* and *Titan*olebias *prognathus*. Among the characteristics that allow recognition of the genus *Titanolebias* are: 1) the presence of over 40 scales in the lateral line, and 2) the pastel yellow to golden color in the center of the scales on the flanks, above the anal fin.

The known distribution of this genus spans a large part of the Río de la Plata basin (excluding the upper and middle Uruguay and Paraná rivers), the headwaters of the Río Mamoré basin, the Laguna Merín basin, and the Atlantic basins in eastern Uruguay, in Argentina, Brazil, Bolivia, Uruguay, and Paraguay (Figure 15).

***Acrolebias* Costa, 2008 (Figure 16)**

Type species: *Cynolebias carvalhoi* Myers, 1947

Etymology: From the Greek "*akro*" = summit or peak (referring to the high-altitude habitats of these fish), "*lebias*" = small fish.

Initially, *Acrolebias* was proposed as a subgenus of *Austrolebias*, including *Acrolebias carvalhoi* and *Amatolebias varzeae*. Later, Loureiro *et al*. (2018) tentatively applied the subgenus *Acrolebias* to *Garcialebias araucarianus* and the species of the "*adloffii* group." Although they did not include *Acrolebias carvalhoi* in their analyses, the species that bears the name *Acrolebias*, they argued that since *Acrolebias araucarianus* is "very similar" and considered close to the species of the "*adloffii* group," the entire group of species could be included in the subgenus *Acrolebias*. As for *Amatolebias varzeae*, they found it closely related to *Amatolebias wichi* rather than *A. carvalhoi* or the "*adloffii* group" species, so they excluded it from that grouping. Despite this work, Lanés *et al*. (2021) chose to include two species they described, *Garcialebias nubium* and *Garcialebias botocudo*, in this subgenus. In our study, we were able to include *Acrolebias carvalhoi* in the analysis based on the available morphological information from Costa's 2010 work, although we did not have genetic sequences available for this species. However, *G. nubium* and *G. botocudo* could not be analyzed because we did not have available material, and these species have not yet been included in phylogenetic analyses.

As a result of our analyses, we found that *A. carvalhoi* is the sister group, meaning it is most closely related, to the species of the genus *Cypholebias* (*C. cinereus* and *C. robustus*). Interestingly, the first known specimens of *Cypholebias cinereus*, collected in Uruguay, were initially identified as *A. carvalhoi* (more details in Serra & Alonso, 2020). Based on these results, we restrict the genus *Acrolebias* to *A. carvalhoi*, which is currently considered the only species belonging to this genus.

Thus, the genus can be recognized, among other characteristics, by having 1) golden flanks with 8 or 9 violet-gray bars in males, 2) separated pelvic fins, 3) the origin of the dorsal fin anterior to the origin of the anal fin in males, and 4) a characteristic number of rays in the dorsal and anal fins and scales in the longitudinal series.

The sole species of this genus is endemic to the upper Iguazu River basin in Brazil (Figure 17), where it has only been collected on a few occasions since its description in 1947, in a highly degraded agricultural area, potentially making it extinct.

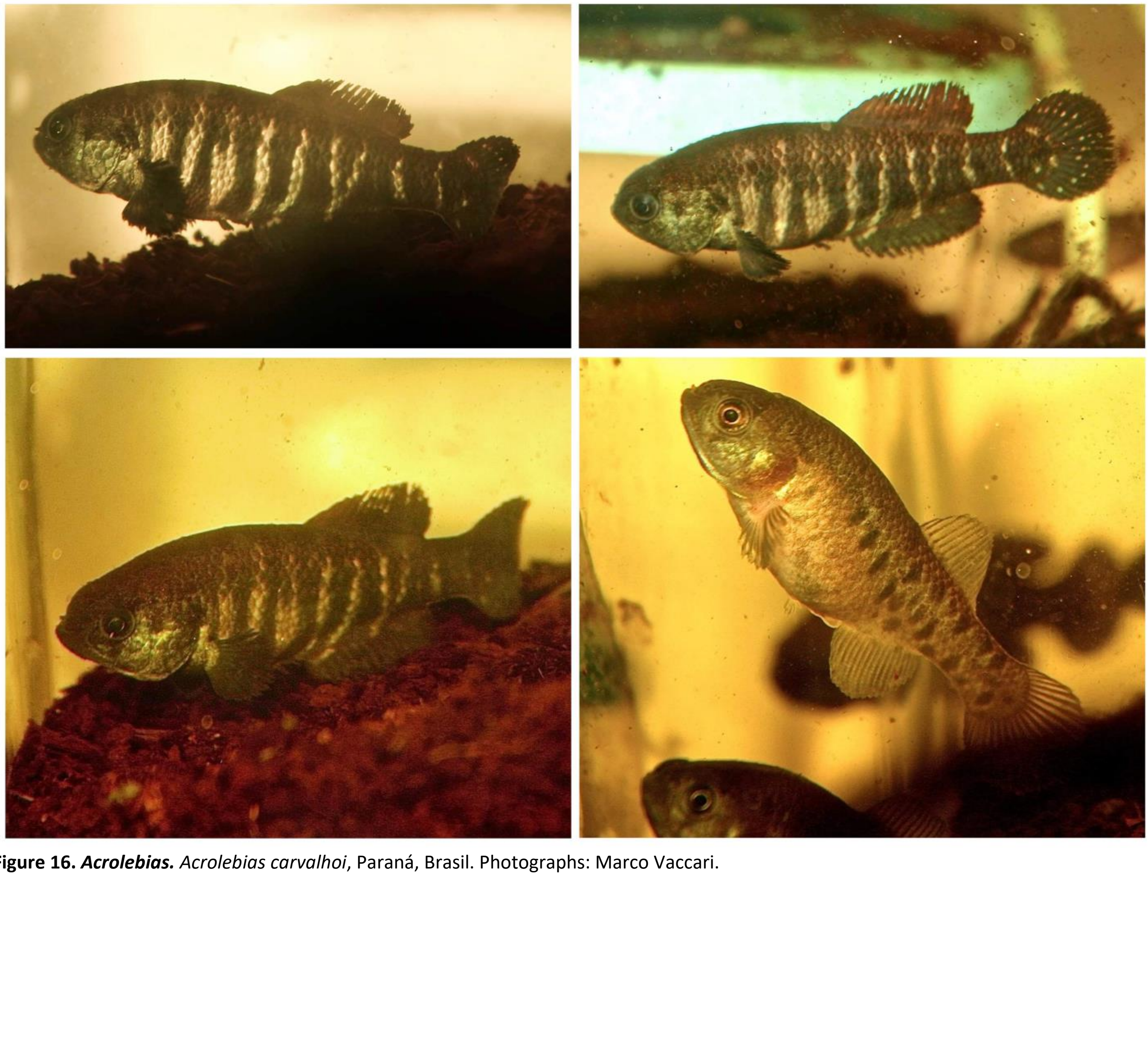

Figure 16. *Acrolebias.* *Acrolebias carvalhoi*, Paraná, Brasil. Photographs: Marco Vaccari.

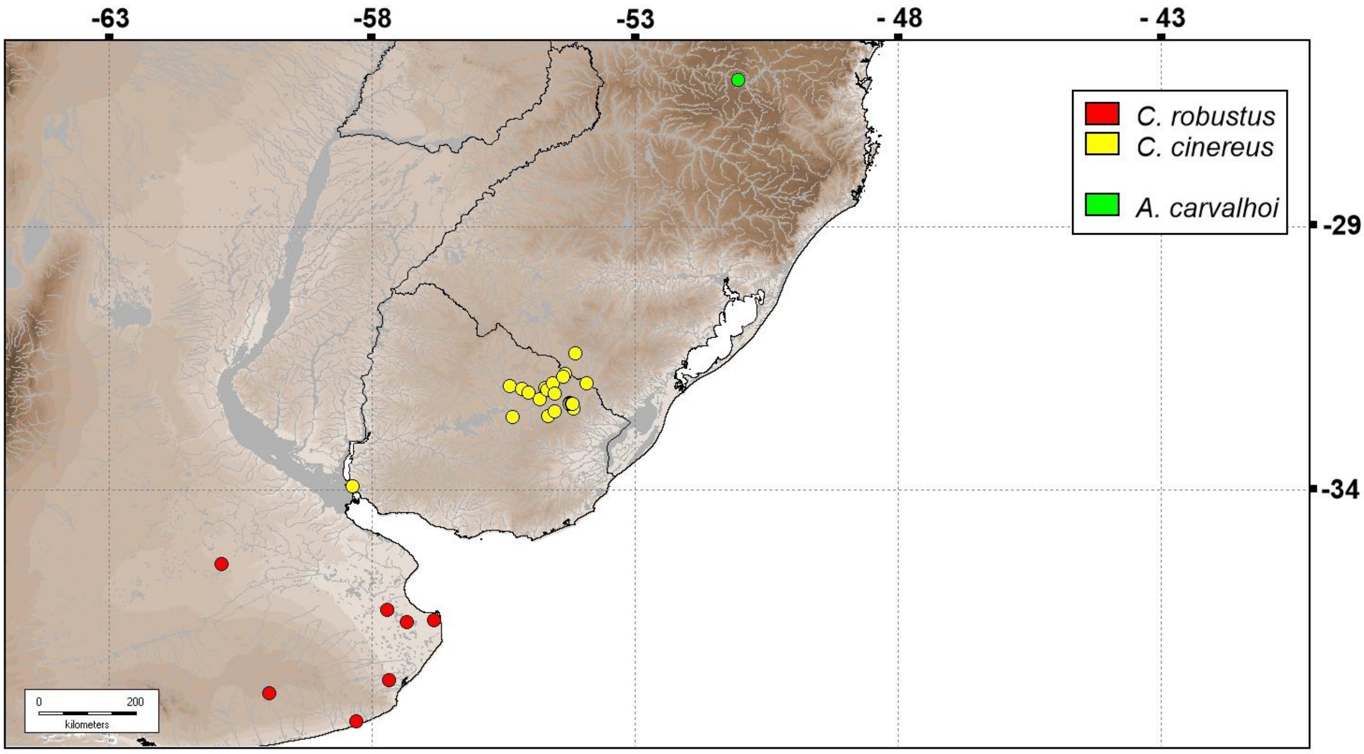

Figure 17. Reported geographic distribution for the species of the genera *Acrolebias* y *Cypholebias.*

Cypholebias **Costa, 2008 (Figure 18)**

Type species: *Cynolebias robustus* Günther, 1883

Etymology: "*kyphos*" = hunchbacked (referring to the curvature of the anterior dorsal region before the dorsal fin in adult specimens), "*lebias*" = small fish.

In 2008, the subgenus *Cypholebias* was created within *Austrolebias* to group four species: *Cypholebias cinereus, C. robustus, C. vazferreirai*, and *C. nonoiuliensis*, with the latter two currently considered synonyms of the former two. Consequently, the genus *Cypholebias* is currently composed of two species: *Cypholebias cinereus* and *Cypholebias robustus*.

This genus can be recognized, among other characteristics, by the following features: 1) fine, irregular light bands on the flanks of males (which may eventually disappear), 2) rounded caudal fin, and 3) 26 to 30 rays in the caudal fin.

The genus *Cypholebias* is distributed in the lower Uruguay River basin in Uruguay, as well as in the basins associated with Laguna Merín in Brazil and Uruguay, and in the basins associated with the Río de la Plata and the Atlantic in the Province of Buenos Aires, Argentina (Figure 17). Being *C. robustus* southernmost seasonal killifish species of the world.

Garcialebias **Alonso *et al*., 2023 (Figure 19)**

Type species: *Cynolebias reicherti* Loureiro & García, 2004

Etymology: The genus name is dedicated to the Uruguayan researcher Dr. Graciela García, who specializes in neotropical fish genetics, particularly in the Rivulidae family. The name "*Garcialebias*" combines her surname with the term "*lebias*," which means small fish in Greek.

The genus *Garcialebias* has a relatively complex taxonomic history, with various studies proposing different names and species groupings based on

Figure 18. ***Cypholebias.*** *Cypholebias cinereus* de (A) Colonia y (B) Cerro Largo, Uruguay. *Cypholebias robustus* (C-D), Buenos Aires, Argentina. Photographs: Pablo Calviño (C, D) and Sebastián Serra (A, B).

different phylogenetic results from morphological and/or molecular data, leading to different interpretations of how to classify these species. Costa (2008) grouped several of them in the "*adloffi* group" within the subgenus *Austrolebias*. García *et al*. (2014) suggest that they are not closely related to the "*bellottii* group" (subgenus *Austrolebias*). Meanwhile, Loureiro *et al*. (2018) classified them in the subgenus *Acrolebias* along with *G*. *araucarianus* and *A. carvalhoi*, although they did not include the latter species in the analysis that carries the name *Acrolebias*, assuming that these species must be related to *A. carvalhoi* because they consider *A. araucarianus* to be "similar" to *A. carvalhoi*. Recently, Lanés *et al*. (2021) included the species *G. araucarianus, G. botocudo, G. nubium* and *A. carvalhoi* in the subgenus *Acrolebias*, separating them from the rest of the species in the "*adloffi* group" that they did not include in that genus. However, their classification was not based on a phylogenetic analysis but rather on the discussion of the presence or absence of certain shared characters between these groups of species.

As a result of our phylogenetic analysis, we found that *Acrolebias carvalhoi* is more closely related to the genus *Cypholebias* than to *G. araucarianus* or the species in the "*adloffi* group". Since *Acrolebias carvalhoi* is the type species of *Acrolebias*, the other species did not have a genus that included them. Therefore, we have created the genus *Garcialebias* to group them. However, the inclusion of *G. botocudo* and *G. nubium* in *Garcialebias* is tentative, as they could not be phylogenetically analyzed, and their inclusion is based on the observation of distinctive characteristics of this genus that we observed in these two species using photographs and descriptions. Nevertheless, including these species in a phylogenetic analysis could generate new hypotheses about their evolutionary relationships and, therefore, their taxonomic classification.

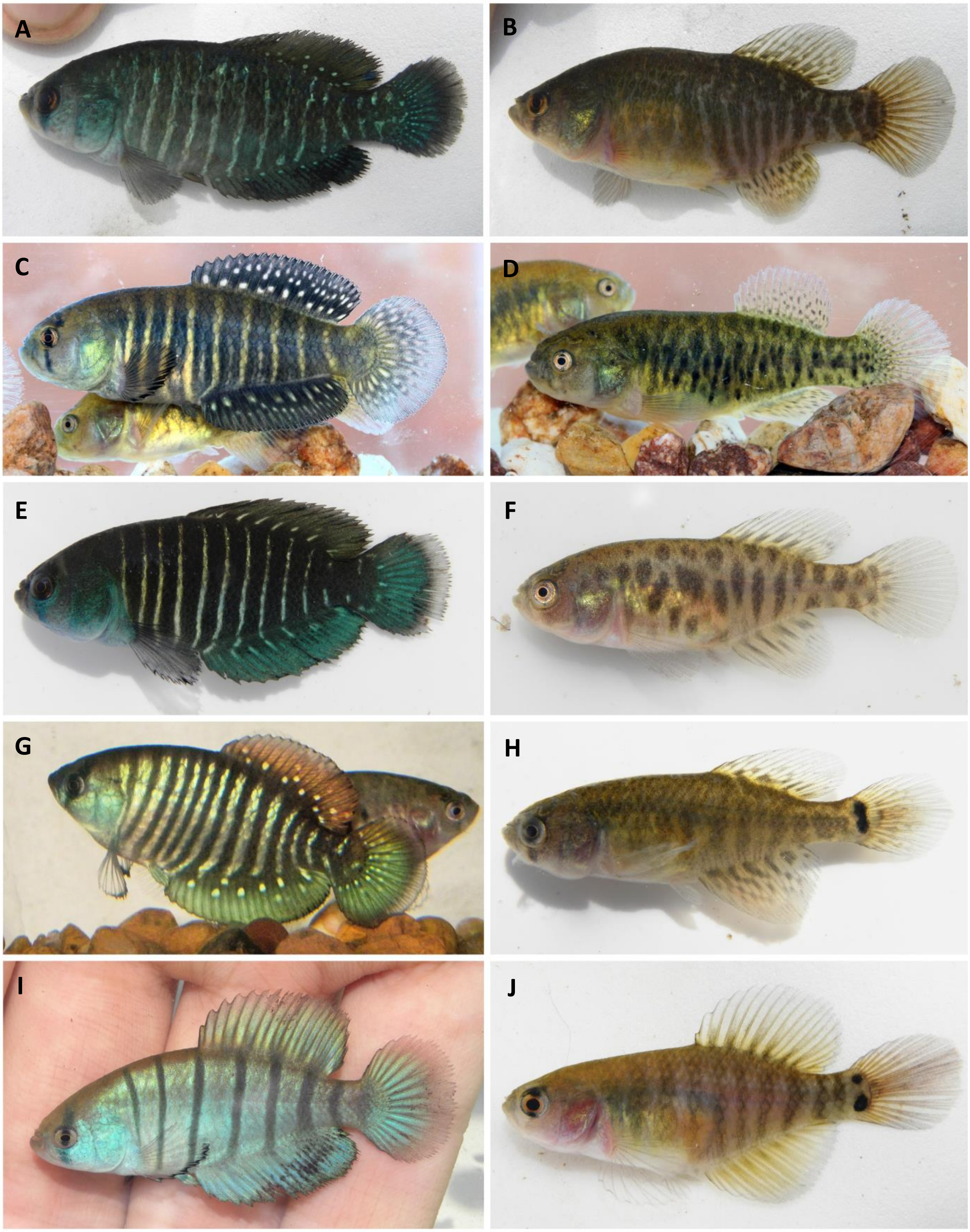

Figure 19. ***Garcialebias***. *Garcialebias viarius* (A-B), Rocha, Uruguay. *Garcialebias nubium* (C-D), Rio Grande do Sul, Brasil. *Garcialebias arachan* (E-F), Río Negro, Uruguay. *Garcialebias charrua* (G-H), Rocha, Uruguay. *Garcialebias reicherti* (I-J) Treinta y Tres, Uruguay. Photographs: Matheus Volcan (C, D) and Sebastián Serra (A, B, E, F, G, H, I, J).

In summary, the genus *Garcialebias* includes 16 species: *Garcialebias adloffi, Garcialebias arachan, Garcialebias araucarianus, Garcialebias bagual, Garcialebias botocudo, Garcialebias charrua, Garcialebias cheffei, Garcialebias lourenciano, Garcialebias minuano, Garcialebias nigrofasciatus, Garcialebias nachtigalli, Garcialebias nubium, Garcialebias pelotapes, Garcialebias pongondo, Garcialebias reicherti* and *Garcialebias viarius*.

This genus can be distinguished from others by a series of characteristics, including: 1) the approximately triangular shape of the anal fin in females, 2) the presence of vertical gray bars on the flanks of males, 3) the absence of iridescent spots on the sides of the male's body, and 4) transparent pectoral fins with a dark margin in males (except in the case of *G. viarius*, which lacks this dark margin).

The genus *Garcialebias* is distributed in several basins, including the Río Negro basin, which belongs to the lower basin of the Río Uruguay, the upper basins of the Río Uruguay and Río Iguazú in Brazil, and the Atlantic basins of Uruguay and the Patos-Merín system of lagoons in Brazil and Uruguay (Figure 20).

Some Final Remarks

The proposed phylogenetic hypothesis is the most comprehensive in terms of the number of species and types and the amount of analyzed information, including 90% of the species, 191 morphological characters, and 10 genes. This has resulted in a highly resolved and robust phylogeny. Of course, scientific knowledge is not immutable, and the incorporation of new evidence (new species, morphological characters, genes, etc.) may lead to some changes in our hypotheses. However, as we have seen in the different scientific studies reviewed here, many species groupings have been resistant to different methodological treatments or sources of information. This indicates that phylogenetic hypotheses tend to stabilize over time as more information accumulates. However, due to the complexity of evolutionary processes, many parts of a phylogeny can be difficult to resolve because they represent complex evolutionary phenomena that are challenging to

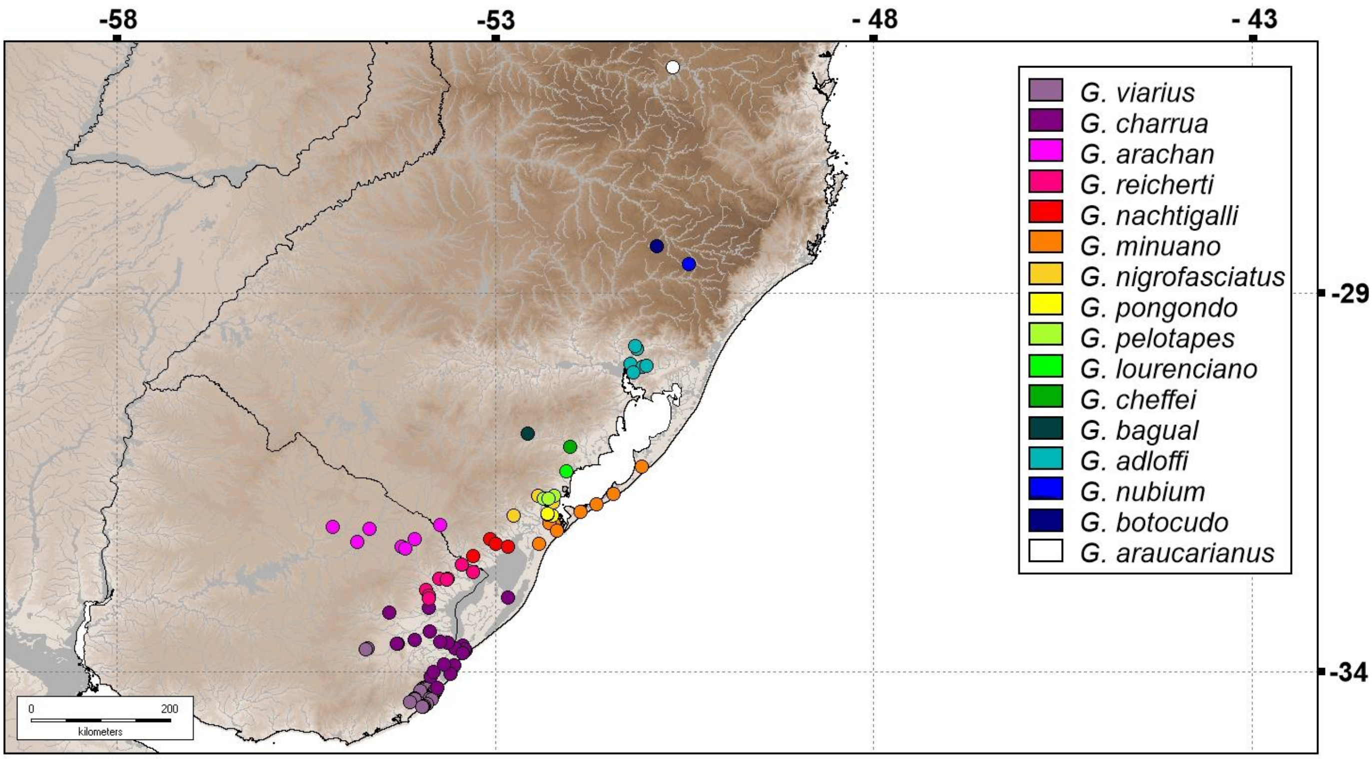

Figure 20. Reported geographic distribution for the species of the genus *Garcialebias.*

model using classical phylogenetic inference methods. For example, various studies show that evolutionary processes often occur with gene flow, and hybridization between species, even distantly related ones, can be a relatively common phenomenon in nature and relevant in evolutionary terms, potentially driving the evolution by generating new variants and species.

As we continue to advance with these types of studies, many new questions arise about how this group of fish has evolved, what geological, ecological, climatological, etc. events have influenced their evolution, and in what ways. We know that there is still much to be explored, but we also believe that this work builds upon the knowledge developed by various researchers over more than a century, from Steindachner to Berg, Vaz-Ferreira, Amato, Costa, Loureiro, Lanés, Volcán, García, and many others. Thanks to them, we can say that today we are standing on the shoulders of giants, and we can look forward with a solid foundation and pose new questions.

Now, many might wonder, what is the use of this type of knowledge? Firstly, it helps us understand the evolutionary relationship between species diversification and changes in geography, basins, and climate over time, enhancing our understanding of evolution, a field known as Biogeography. In the specific case of seasonal fish, as they live in highly specific environments and have relatively low dispersal capabilities, they often exhibit a strong evolutionary relationship with geological changes in geography, such as changes in basin morphology and connectivity, marine incursions, etc. Particularly when studying the relationship between genetic structure and geography, these studies are referred to as phylogeographic studies. Phylogeography utilizes genetic techniques to reconstruct the evolutionary history and patterns of dispersal, isolation, etc., of a lineage (which can be populations of a species or groups of populations of closely related species, for example) through time and space. In the case of South American killifish, there are several research groups dedicated to this topic in Argentina, Brazil, and Uruguay.

On the other hand, new phylogenetic hypotheses can have a significant impact on biodiversity conservation policies, land management, and the planning of protected areas. Geographical areas with higher phylogenetic diversity are potentially more important for conservation than those with lower diversity. For example, if an area is inhabited by one species of *Garcialebias*, one species of *Titanolebias*, and one species of *Acantholebias*, it will be a priori more valuable from a conservation perspective than an area that has three species of *Garcialebias*. When not all areas can be conserved due to agricultural expansion, urbanization, or other reasons, these criteria can be useful in deciding where to focus our resources and conservation efforts. In the case of South American seasonal fish, these types of studies (e.g., Costa, 2012; Garcez *et al*., 2022) are still in early stages of development and are mainly led by researchers from Brazil. It is crucial to continue investing in research like these as they can provide valuable information for the conservation of these species and the protection of their habitats. Furthermore, this approach can be applied to other groups of species worldwide and be of great help in making decisions regarding global biodiversity management.

It is important to remember that changes in organism names are not arbitrary or meaningless. On the contrary, they reflect the changes that occur in the scientific knowledge associated with those organisms. The way we name living beings attempts to reflect how these beings are related from an evolutionary perspective. As a result, new phylogenetic hypotheses often imply changes in nomenclature, as has been the case with our work.

While the decision to elevate subgeneric groups to genera may have a subjective component, we believe that this change helps visualize the existence of these evolutionarily defined and relatively homogeneous groupings, both morphologically and genetically. This is useful for everything mentioned in the previous paragraphs, including decision-making on conservation and protection of biodiversity in priority areas with higher phylogenetic diversity. For example, one of the ponds that is a study area for our research group contained three species belonging to the genus *Austrolebias*. Now, we can say that this pond has greater richness in terms of generic diversity since, in addition to the genus *Austrolebias*, the genera *Argolebias* and *Titanolebias* are also present (*Austrolebias bellottii*, *Argolebias nigripinnis*, and *Titanolebias elongatus*). In conclusion, continuing research in these approaches will allow us to advance in the understanding and conservation of global biodiversity.

Acknowledgments

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