# Under the surface: what we know about the threats to subterranean fishes in Brazil

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The present work brings information on threats to the subterranean fishes in Brazil. Currently, at least 36 species are known, 22 of which are already formally described. Endemism is the rule for most of them. Regarding their conservation, these fishes are in general considered threatened: and most of the already formally described species are included in national lists of threatened fauna, and only four of them are included in the global list of the IUCN. Regarding habitats, Brazilian subterranean fishes occur in alluvial sediments (part of the hyporheic zone), shallow base-level streams, flooded caves, lakes in the water table, upper vadose tributaries, and epikarst aquifers. We detected 11 main threats, mainly related to agriculture, pasture, and hydroelectric plans, but unmanaged tourism and pollution are also significant threats. Two threats affect a high number of species (physical change of the habitat and food restriction). The river basins with the higher number of identified threats are the upper Tocantins (eight) followed by the upper Paraguaçu (six). Effective proposals to protect this neglected component of the Brazilian biodiversity are still scarce, such as monitoring projects and their function in the subterranean communities, besides education projects aiming to develop public awareness.

Keywords: Conservation, Human impacts, IUCN, Subterranean fishes, Threats.

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O presente trabalho traz informações sobre as ameaças aos peixes subterrâneos no Brasil. Atualmente, são conhecidas pelo menos 36 espécies, 22 das quais já foram formalmente descritas. O endemismo é a regra para a maioria destas espécies. Em relação à conservação, esses peixes são em geral considerados ameaçados: a maioria das espécies já descritas está incluída em listas regionais de fauna ameaçada e apenas quatro delas estão incluídas na lista global da IUCN. Em relação aos habitats, os peixes subterrâneos brasileiros ocorrem em sedimentos aluviais (parte da zona hiporreica), riachos de nível de base, cavernas inundadas, lagos no lençol freático, tributários vadosos superiores e aquíferos no epicarste. Detectamos onze ameaças principais, a maioria relacionada à agricultura, pecuária e projetos hidrelétricos, entretanto, turismo sem planos de manejo e poluição também representam ameaças significativas. Duas ameaças afetam um grande número de espécies: a mudança física do habitat e a restrição de alimento. As bacias hidrográficas com o maior número de ameaças identificadas são a do alto Tocantins (oito) seguida pela do alto Paraguaçu (seis). Propostas eficazes para proteger esse componente negligenciado da biodiversidade brasileira são ainda escassos, como os projetos de monitoramento e sua função nas comunidades subterrâneas, além de projetos relacionados à educação, visando a sensibilização do público.

Palavras-chave: Ameaças, Conservação, Impactos Humanos, IUCN, Peixes subterrâneos.

## INTRODUCTION

The subterranean or hypogean environment comprises a series of interconnected subsurface spaces of variable dimensions, from interstices of a few millimeters to very large galleries and caves, formed in solid rock and filled with water or air (Juberthie, 2000a). When compared to surface or epigean habitats, subterranean habitats (including non-cave habitats) show particularities that, together, form a specific kind of selective regime, which may constrain potential colonizer organisms. Examples are the permanent absence of light in the deeper zones and, consequently, of primary producers, generally resulting in conditions of food scarcity and dependence of allochthonous input (originated in epigean habitats). Another characteristic is the tendency towards climatic stability as a consequence of the buffer effect of the surrounding rocks (Culver, Pipan, 2009).

It is widely accepted that organisms with abilities to explore dark and food-scarce habitats are potential colonizers of subterranean environments (Poulson, White, 1969; Culver, Pipan, 2009). Organisms that rely on sensory modalities other than vision, such as chemo- and mechanoreception, with a generalist and/or detritivorous diet, could be more successful in colonizing subterranean environments and establishing hypogean populations. Trajano (2012) proposed a classification of subterranean organisms based on the concept of sink-source populations. This classification defines as trogloxenes, organisms that have source populations in the epigean environment but use subterranean resources (*e.g.*, bats); troglophiles, organisms that have source populations in both hypogean and epigean environments (*e.g.*, several populations of fishes, spiders, and crickets); and troglobites, organisms that have source populations exclusively in the hypogean environment (*e.g.*, several species of fishes, crustaceans, insects and others). In Brazil, troglobitic and troglophilic populations of fishes were already identified (*e.g.*, Bichuette, Trajano, 2003, 2021; Trajano, Bichuette, 2010; Ratton *et al*., 2018; Rabello, 2021); no trogloxen fishes are reported in Brazil.

It is a worldwide consensus that subterranean habitats, as well as their communities, are highly singular, fragile, and represent one of the most threatened environments in the world (Elliott, 2005; Culver, Pipan, 2019; Mammola *et al*., 2019). Troglobites, in particular, are intrinsically fragile and vulnerable to environmental changes, given their small populations with low resilience (Culver, Pipan, 2009). All troglobitic species should be protected by law and included in at least the Vulnerable category (VU) of IUCN (International Union for Conservation of Nature), an idea that has been advocated by several authors (*e.g*., Juberthie, 2000b; Bichuette, Trajano, 2010, 2021; Fernandes *et al*., 2016; Trajano *et al*., 2016; Gallão, Bichuette, 2018; Culver, Pipan, 2019; Mammola *et al*., 2019; Bichuette, 2021).

In Brazil, the intense exploration of mineral resources, agriculture and pasture activities, urban expansion, and pollution of aquifers have grown in the years following the publication of the Decree 6640 (Brasil, 2008) that classifies caves by their levels of relevance (see Gallão, Bichuette, 2018). Only those caves classified as of maximum relevance with basis on a list of attributes are considered prone to be effectively protected by the Brazilian legislation. This has led to the suppression, in some cases, of entire massifs, whether of limestone or other lithologies (*e.g.*, sandstone, igneous, iron ore) and consequently to expressive losses of subterranean biodiversity (Gallão, Bichuette, 2012, 2018). Attributes that classify a given cave as of maximum relevance, therefore assuring (in theory) its full protection according to the Decree, are: it harbors rare troglobitic species, includes unique ecological interactions, or is essential for the preservation of relict, endemic, or threatened troglobites – in this case, the species must be included in official lists (regional, national or global) of threatened fauna, an important attribute that denotes total protection to caves (Gallão, Bichuette, 2012).

Furthermore, it is a worldwide consensus that subterranean habitats are highly singular, and one of the most threatened in the world (Culver, Pipan, 2019). Unfortunately, other types of Brazilian subterranean habitats beyond caves, such as the hyporheic zone (defined as an active ecotone delimited superiorly by channel water and inferiorly by underground water, constituted by particles of the riverbed; Mugnai *et al*., 2015), MSS (*Milieu Souterrain Superficiel*, defined as the upper zone of the rock, forming a subterranean network of empty air-filled voids of rock fragments; Juberthie, 2000a), and even aquifers, are not considered in the Decree 6640.

The Brazilian subterranean ichthyofauna is remarkable due to its phylogenetic diversity, at both the family and genus levels, when compared to subterranean ichthyofaunas of other regions of the world. The countries with the higher number of troglobitic fish species are China (more than 79 species) and Brazil (36 species, see below) (Bichuette, 2021). However, the phylogenetic diversity in China, 10 genera in four families (Niemiller *et al*., 2019) is smaller when compared to Brazil, with 13 genera in seven families (see below). This is somehow expected given the fact that Brazil is a megadiverse country and houses a substantial part of the world's freshwater ichthyofauna, which includes potential colonizers of subterranean habitats. The vast majority of the Brazilian troglobitic ichthyofauna belongs to the order Siluriformes (catfishes), mainly from the families Trichomycteridae and Heptapteridae (Trajano, Bichuette, 2010; Bichuette, 2021).

By considering also the several Brazilian fish species that have troglophilic populations, about 50 (Bichuette, Trajano, 2003; Trajano, Bichuette, 2010; Bichuette, 2021), the urge to protect subterranean habitats that house these populations is evident. Considering this concerning scenario, we present herein updated data about the already identified threats to this particular ichthyofauna, with a discussion on existing and persisting gaps of distribution data.

## MATERIAL AND METHODS

We evaluated the threats to the 36 subterranean species reported herein (see Tabs. 1–2). The undescribed species (14 from 36) are under study by specialists, and their status as new taxon is corroborated by morphological studies, with detection of diagnosis.

A descriptive analysis of threats was considered through search in data published in literature concerning the conservation of cavefishes, including the information of the original description of species: articles in journals, books, chapter books, thesis and dissertations, and proceedings of national and international meetings (*e.g.*, Bichuette, 2008; Trajano, 2008a,b; Bichuette, Trajano, 2010, 2015, 2021; Gallão, Bichuette, 2012, 2018; Borghezan, 2013; ICMBio, 2018). In addition, we included personal observations collected during fieldwork in several regions of Brazil for the last 18 years.

These data are presented in detail in the maps of the known subterranean fish species occurring in Brazil, including the ones not yet described, tables, and graphs, considering the threats for each taxon. Maps were elaborated using the software Quantum GIS (QGIS, version 3.6.0, https://www.qgis.org/en/site), and shapefiles from MapBiomas (version 5.0, https://mapbiomas.org).

## RESULTS

Brazil houses a rich subterranean ichthyofauna compared to China and Mexico, both megadiverse countries for subterranean fishes. We have reported 36 species so far, 22 of which have already been formally described (Tab. 1). Brazilian subterranean fishes are found in six basins (Figs. 1-2) and eight federated states (Tab. 1). Considering the occurrences, we observe several gaps of distribution, for example, the Amazon basin and the upper rio Paraguai (Fig. 1) and some regions with high richness and still high potential for troglobitic species, such as the northeastern region of Goiás, Central Brazil, and the southwestern and northern regions of Bahia (Figs. 1 and 3). The bias in collection explains these gaps since northeastern of Goiás was intensively prospected in search of cavefish in the last 15 years by our team. Endemism is the rule for most of them, with at least 20 species occurring in one single cave or cave system (*i.e.*, one or more caves extending continuously between sinkholes and resurgences) or in non-cave habitats, such as hyporheic zone.

**TABLE 1** | List of the currently known Brazilian subterranean fishes species occurring in Brazil, their basins of occurrence, type of habitats / number of localities (caves or non-cave habitats). * Undescribed species with references.

| Species | River basin | State | Habitat / number of caves or localities |
|---|---|---|---|
| *Stygichthys typhlops* Brittan & Böhlke, 1965 | Middle rio São Francisco | Minas Gerais | Phreatic / 1 |
| *Eigenmannia vicentespelaea* Triques, 1996 | Upper rio Tocantins | Goiás | Base-level stream / 2 |
| *Ancistrus cryptophthalmus* Reis, 1987 | Upper rio Tocantins | Goiás | Base-level stream / 4 |
| *Ancistrus formoso* Sabino & Trajano, 1997 | Upper rio Paraguai | Mato Grosso do Sul | Flooded caves / 3 |
| *Ancistrus* sp. "Bodoquena"* (Trajano, Bichuette, 2010; Borghezan, 2013) | Upper rio Paraguai | Mato Grosso do Sul | Flooded cave / 2 |
| *Aspidoras mephisto* Tencatt & Bichuette, 2017 | Upper rio Tocantins | Goiás | Base-level stream / 2 |
| *Trichomycterus itacarambiensis* de Pinna & Trajano, 1996 | Middle rio São Francisco | Minas Gerais | Base-level stream / 1 |
| *Trichomycterus dali* Rizzato, Costa-Jr, Trajano & Bichuette, 2011 | Upper rio Paraguai | Mato Grosso do Sul | Flooded caves / 3 |
| *Trichomycterus rubbioli* Bichuette & Rizzato, 2012 | Middle rio São Francisco | Bahia | Upper Phreatic (cave) /1 |
| *Trichomycterus* sp. "ramalho"* (Bichuette, 2021) | Middle rio São Francisco | Bahia | Base-level stream / 1 |
| *Trichomycterus* sp. "iu iu"* (Bichuette, 2021) | Middle rio São Francisco | Bahia | Upper Phreatic (cave) / 1 |
| *Ituglanis passensis* Fernández & Bichuette, 2002 | Upper rio Tocantins | Goiás | Base-level stream / 1 |
| *Ituglanis bambui* Bichuette & Trajano, 2004 | Upper rio Tocantins | Goiás | Vadose tributary / 1 |
| *Ituglanis epikarsticus* Bichuette & Trajano, 2004 | Upper rio Tocantins | Goiás | Epikarst / 1 |
| *Ituglanis ramiroi* Bichuette & Trajano, 2004 | Upper rio Tocantins | Goiás | Vadose tributary /1 |
| *Ituglanis mambai* Bichuette & Trajano, 2008 | Upper rio Tocantins | Goiás | Base-level stream / 1 |
| *Ituglanis boticario* Rizzato & Bichuette, 2015 | Upper rio Tocantins | Goiás | Base-level stream / 1 |
| *Ituglanis* sp. "terra ronca"* (Bichuette, 2021) | Upper rio Tocantins | Goiás | Vadose tributary / 1 |
| *Ituglanis* sp. "posse"* (Bichuette, 2021) | Upper rio Tocantins | Goiás | Upper Phreatic (cave) / 1 |
| *Ituglanis* sp. "canastra"* (Bichuette, 2021) | Upper rio São Francisco | Minas Gerais | Base-level stream |
| *Glaphyropoma spinosum* Bichuette, de Pinna & Trajano, 2008 | Upper rio Paraguaçu | Bahia | Base-level stream / 8 |
| *Copionodon* sp. "igatu"* (Bichuette, 2021) | Upper rio Paraguaçu | Bahia | Base-level stream / 3 |
| *Pimelodella kronei* (Miranda Ribeiro, 1907) | Upper rio Ribeira | São Paulo | Base-level stream / 7 |
| *Pimelodella spelaea* Trajano, Reis & Bichuette, 2007 | Upper rio Tocantins | Goiás | Vadose tributary / 1 |
| *Pimelodella* sp. "açungui"* (Bichuette, 2021) | Upper rio Ribeira | São Paulo | Base-level stream / 1 |
| *Rhamdia enfurnada* Bichuette & Trajano, 2005 | Middle rio São Francisco | Bahia | Base-level stream / 1 |
| *Rhamdia* sp. "Bodoquena"* (Trajano, Bichuette, 2010; Borghezan, 2013) | Upper rio Paraguai | Mato Grosso do Sul | Base-level stream / 2 |
| *Rhamdiopsis krugi* Bockmann & Castro, 2010 | Upper rio Paraguaçu | Bahia | Upper Phreatic (cave) / 12 |
| *Rhamdiopsis* sp. "gonçalo"* (Trajano, Bichuette, 2010) | Middle rio São Francisco | Bahia | Upper Phreatic (cave) / 1 |
| *Rhamdiopsis* sp. "cordisburgo"* (Trajano, Bichuette, 2010) | Middle rio São Francisco | Minas Gerais | Base-level stream / 1 |
| *Rhamdiopsis* sp. "ramalho"*, two populations/ morphotypes (Bichuette, 2021) | Middle rio São Francisco | Bahia | Upper Phreatic (cave) / 1 and 1 |
| *Rhamdiopsis* sp. "caatinga"* (Bichuette, 2021) | Middle rio São Francisco | Bahia | Upper Phreatic (cave) / 1 |
| *Phenacorhamdia* sp. "posse"* (Bichuette, 2021) | Upper rio Tocantins | Goiás | Upper Phreatic (cave) / 1 |
| *Phreatobius cisternarum* Goeldi, 1905 | Amazon basin | Amapá; Pará | Hyporheic (alluvium) / 6 |
| *Phreatobius dracunculus* Shibatta, Muriel-Cunha & de Pinna, 2007 | Amazon basin | Rondônia | Hyporheic (alluvium) / 1 |
| *Phreatobius sanguijuela* Fernández, Saucedo, Carvajal-Vallejos & Schaefer, 2007 | Amazon basin | Rondônia | Hyporheic (alluvium) / 2 |

In relation to habitats, the Brazilian subterranean fishes occur from alluvial sediments (which are part of the hyporheic zone) to shallow base-level streams, flooded caves, upper vadose tributaries, lakes in the water table, and the epikarst (Tab. 1; Fig. 4). The vadose tributaries are located in the unsaturated zone and extend from the top of the ground surface to the water table. Epikarst is the upper part of the vadose zone and is defined as the heterogeneous interface between unconsolidated material, (such as sediment and soil), and altered rock, partially saturated with water and capable of delaying or storing and locally rerouting vertical infiltration to the deeper, phreatic zone of the karst aquifer (Jones *et al*., 2004).

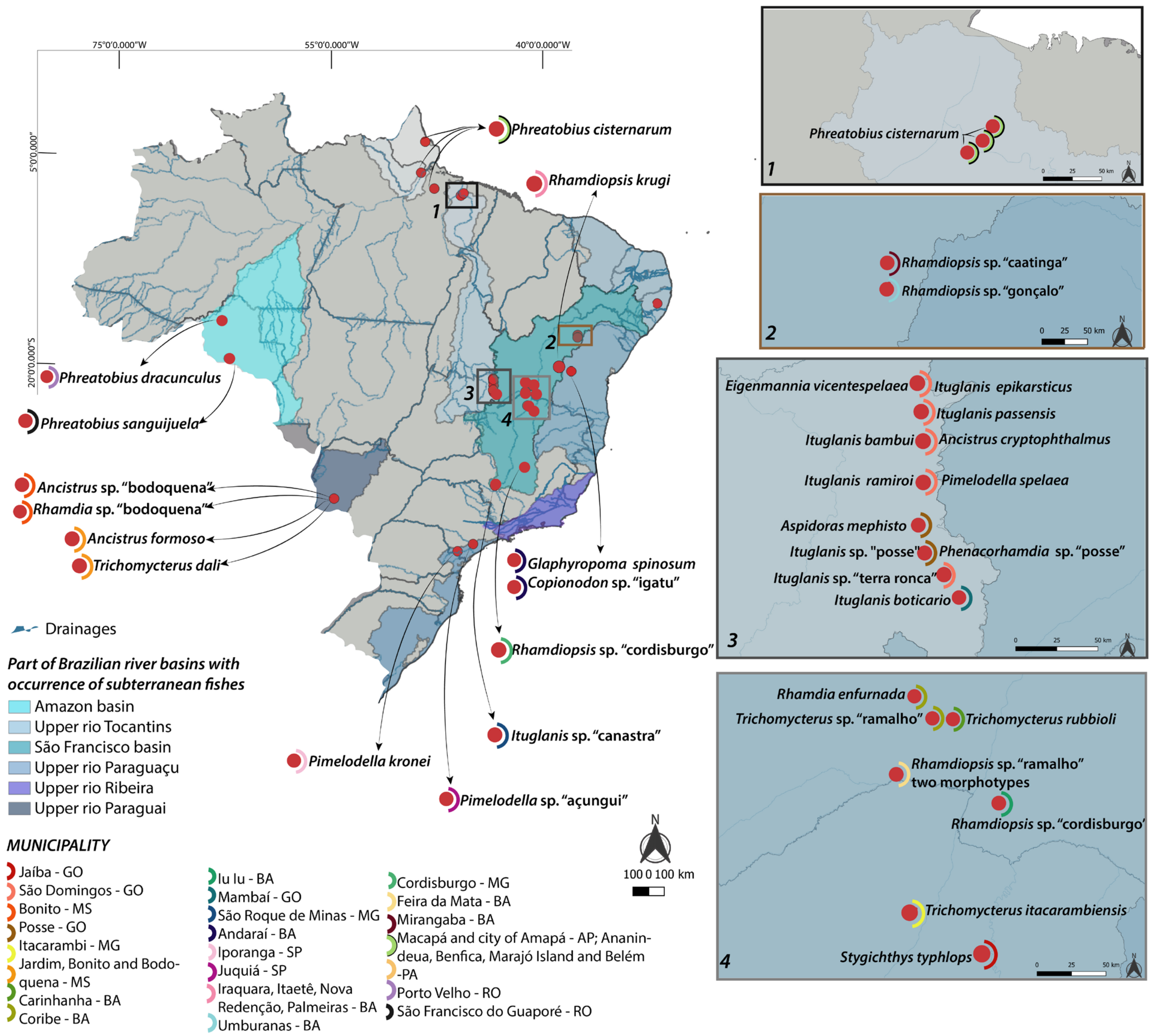


**FIGURE 1** | Map of Brazil showing the distribution of the subterranean fish species according to the basins included in the country's territory.

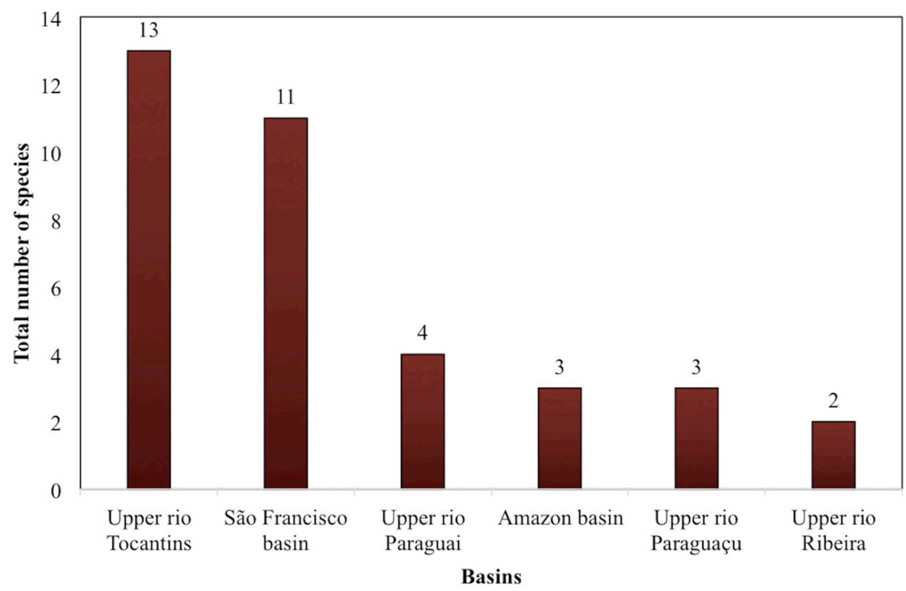


**FIGURE 2 |** Distribution of Brazilian subterranean fish species according to basin.

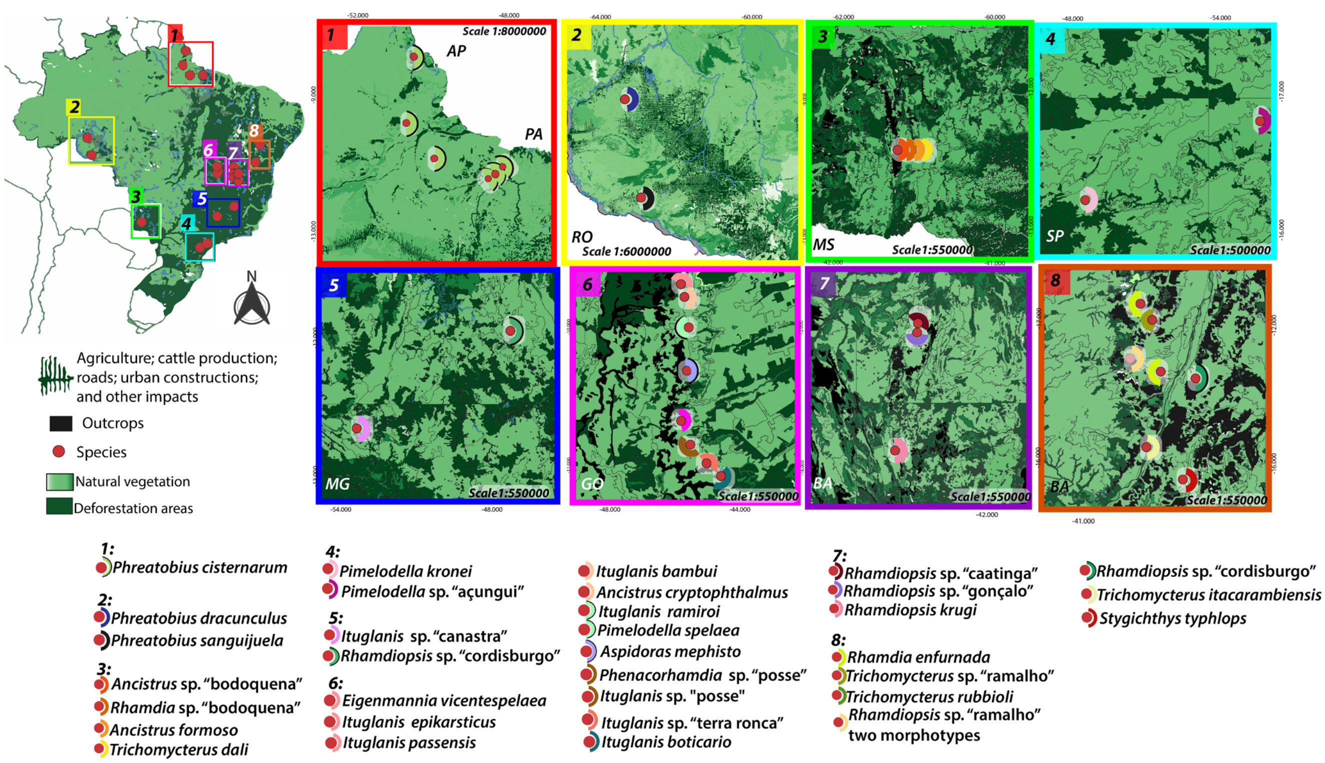


**FIGURE 3 |** Detailed maps showing the distribution of Brazilian subterranean fishes in different federated states, including vegetation and impacts nearby. AP, Amapá State; PA, Pará State; RO, Rondônia State; MS, Mato Grosso do Sul State; BA, Bahia State; SP, São Paulo State, MG, Minas Gerais State; GO, Goiás State. Outcrops, landscape areas with rocks and potentially cave occurrences.

Most species occur in shallow base-level streams, but the occurrence of species in singular habitats is notable. One remarkable species, *Ituglanis epikarsticus* Bichuette & Trajano, 2004 (Fig. 5A) is so far the only subterranean fish species in the world known to occur in the epikarst (Culver, Pipan, 2009; Bichuette, 2021), which emphasized the urge to effectively protect the cave of its occurrence, Lapa do São Mateus, a touristic cave of northeastern Goiás. Some cavefish habitats in Bahia state are fed by aquifers, highly impacted by lowering of the phreatic level, that is observed for two new Heptapteridae catfishes, highly specialized and under threat (*e.g.*, *Rhamdiopsis* sp. "caatinga", Fig. 5B). In the case of the subterranean catfish species of the genera *Glaphyropoma* de Pinna, 1992 and *Copionodon* de Pinna, 1992 (Trichomycteridae: Copionodontinae), the streams pass through sandstone caves associated with quartzites and conglomerates, representing the only record of troglobitic fishes in this type of lithology in Brazil (Bichuette *et al*., 2008). Most Brazilian subterranean fishes live in habitats accessible through caves, except for *Stygichthys typhlops* Brittan & Böhlke, 1965 (Fig. 5C) and species of *Phreatobius* Goeldi, 1905, which are always sampled in artificial wells of karst water outcrops (Moreira *et al*., 2010) or submerged litter banks in the hyporheic zone (Muriel-Cunha, Pinna, 2005; Ohara *et al*., 2016), respectively. Such fragile environments are rarely reported in the literature.

Sixteen subterranean fish species were evaluated in the most recent edition of the Brazilian Red List (ICMBio, 2018). Nine were classified as Vulnerable (VU), five as Endangered (EN), two as Critically Endangered (CR), one as Least Concern (LC), and one as Data Deficient (DD) (Tab. 2). Besides these 16 species, which are under re-evaluation in the Red List workshops, four additional species are under evaluation for the first time (*Aspidoras mephisto* Tencatt & Bichuette, 2017, *Ituglanis boticario* Rizzato & Bichuette, 2015, *Phreatobius sanguijuela* Fernandez, Saucedo, Carvajal-Vallejos & Schaefer, 2007, and *Rhamdia enfurnada* Bichuette & Trajano, 2005, Tab. 2). In the global

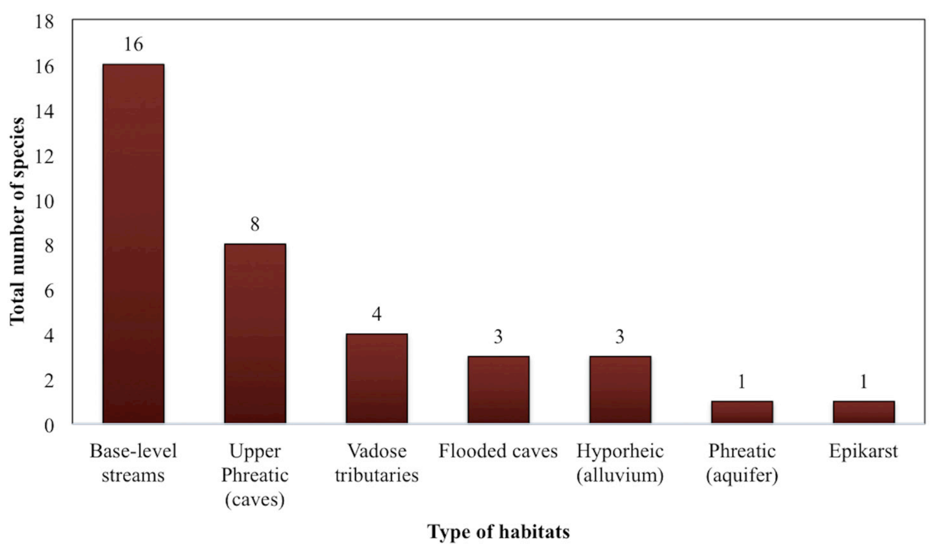


**FIGURE 4** | Distribution of Brazilian subterranean fish species according to the type of habitat.

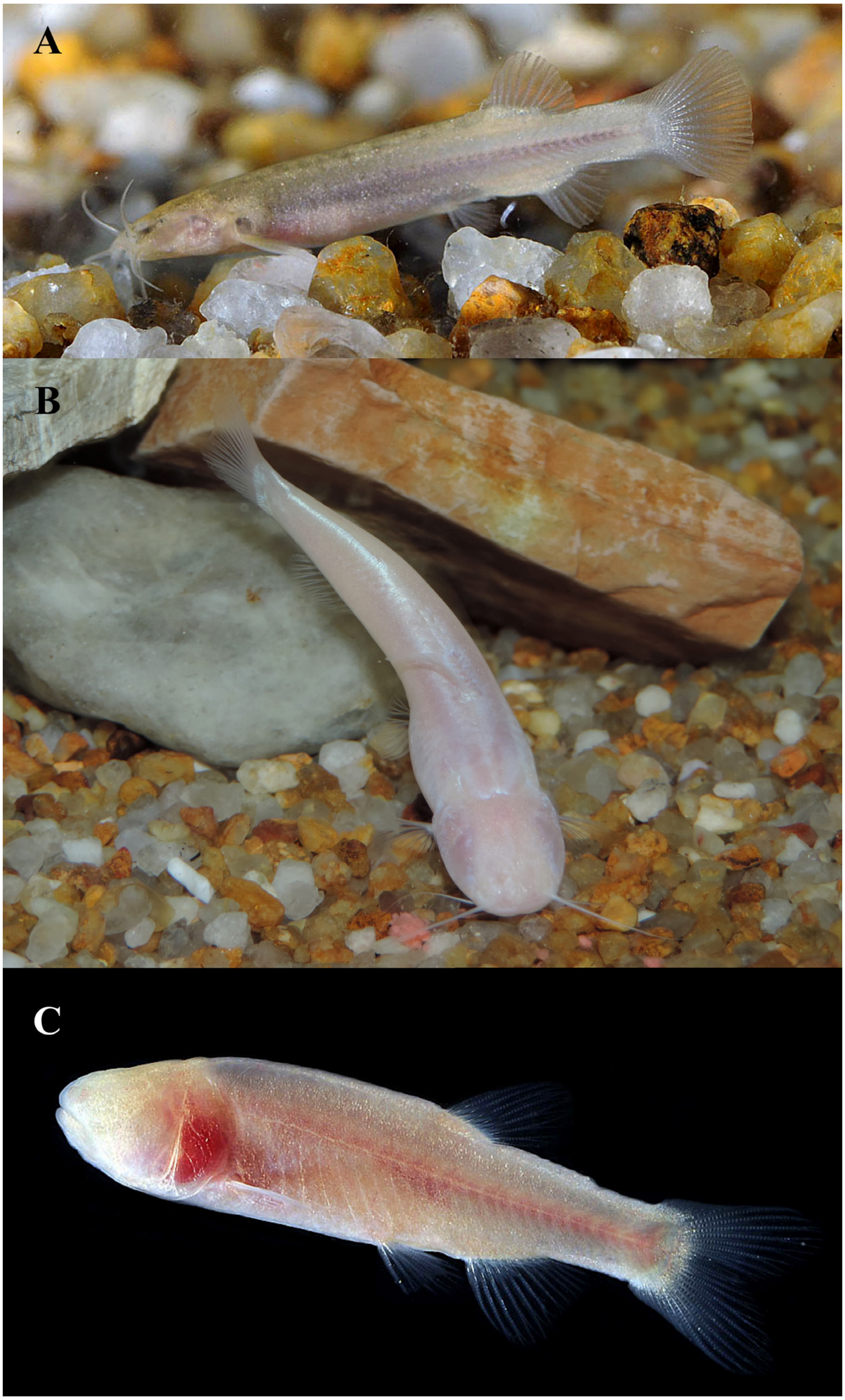


**FIGURE 5 |** **A.** *Ituglanis epikarsticus* from the Lapa do São Mateus cave, São Domingos, Goiás State, live specimen, 28 mm SL; Photo: Adriano Gambarini. **B.** *Rhamdiopsis* sp. "caatinga", live specimen, 59 mm SL; Photo: Jonas Eduardo Gallão. **C.** *Stygichthys typhlops* from the Jaíba region, Minas Gerais State, live specimen, 25 mm SL, specimen captured in July 2008; Photo: Adriano Gambarini. All specimens were photographed in an aquarium at Laboratório de Estudos Subterrâneos, Universidade Federal de São Carlos.

Red List of IUCN, only four species are included, three classified as DD (*Pimelodella kronei* (Miranda Ribeiro, 1907), *Phreatobius cisternarum* Goeldi, 1905, and *Stygichthys typhlops*) and one as CR (*Phreatobius sanguijuela*).

In total, eleven threats were identified, related to at least 15 causes (Figs. 3 and 6; Tab. 2). The main threats to their areas of occurrence are consequences of human activities related to agriculture, pasture, and hydroelectrical plans (Fig. 6). These activities affect different subterranean aquatic habitats, from shallow base-level streams to vadose tributaries, epikarst, and aquifers (Fig. 2; Tab. 2). The main consequences are alterations in the water table (level/volume and pollution) and the physicochemical characteristics of the habitats (pH and alterations and siltation/destruction of microhabitats), causing fragmentation and chemical disturbance. In addition, mining activities can be extremely harmful to this particular ichthyofauna, since can alter the physical characteristics of the habitats. Unmanaged touristic activities also represent an important threat, since can, besides alter the physical habitat, introduce species and even diseases.

The threats that affect more species are the physical change of the habitat (21 species), followed by the food restriction (14 species). The physical change of habitat is the alteration of microhabitats, such as shelters and sites for reproduction (Tab. 2); the food restriction is related to the alterations in the landscape harboring the caves and other subterranean habitats, impacting the input of food for the fish species (Tab. 2).

The basin with more threats is the upper rio Tocantins (eight) followed by upper rio Paraguaçu (six), being the four remaining river basins with two or three threats per each (Fig. 7).

Sixteen taxa (including described and undescribed ones) occur in caves inside Conservation Units (National/State Parks or Environmental Protection Areas/APA) or their boundaries, with minimal legal protection (Tab. 2).

**TABLE 2 |** Brazilian subterranean fishes: threats, possible causes, IUCN categories, and occurrence in Conservation Units.

| Species | Threats | Possible causes | IUCN Brazilian Red List (ICMBio/MMA, 2018) | IUCN Global Red List | Conservation Unit |
|---|---|---|---|---|---|
| *Stygichthys typhlops* Brittan & Böhlke, 1965 | Lowering of the aquifer; physical change of the habitat | Artesian wells for fruit irrigation; climate change | Endangered (EN) | Deficient Data (DD) | Not included |
| *Eigenmannia vicentespelaea* Triques, 1996 | Lowering of the base-level stream; siltation of the subterranean drainage; tourism | Large scale agriculture and irrigation projects (in the headwaters); deforestation of headwaters; unmanaged tourism; climate change | Vulnerable (VU) | Not included | Terra Ronca State Park |
| *Ancistrus cryptophthalmus* Reis, 1987 | Lowering of the base-level streams; tourism | Large scale agriculture and irrigation projects (in the headwaters); deforestation of the headwaters; unmanaged tourism; climate change | Endangered (EN) | Not included | Terra Ronca State Park |
| *Ancistrus formoso* Sabino & Trajano, 1997 | Pollution; physical change of the habitat | Use of pesticides for agriculture; mining for cement production | Vulnerable (VU) | Not included | Limits of the Serra da Bodoquena National Park |

**TABLE 2 |** (Continued)

| Species | Threats | Possible causes | IUCN Brazilian Red List (ICMBio/MMA, 2018) | IUCN Global Red List | Conservation Unit |
|---|---|---|---|---|---|
| *Ancistrus* sp. "bodoquena" | physical change of the habitat | Deforestation; mining projects for cement production | Not included | Not included | Not included |
| *Aspidoras mephisto* Tencatt & Bichuette, 2017 | Pollution; physical change of the habitat; food restriction | Use of pesticides for agriculture; discharge of domestic sewage; mining projects for cement production; deforestation of cave surroundings | Not included | Not included | Not included |
| *Trichomycterus itacarambiensis* de Pinna & Trajano, 1996 | Physical change of the habitat; food restriction | Dams inside the cave for water exploration; climate change | Critically Endangered (CR) | Not included | Limits of the Cavernas do Peruaçu National Park |
| *Trichomycterus dali* Rizzato, Costa-Jr, Trajano & Bichuette, 2011 | Physical change of the habitat; pollution | Mining projects for cement production; water exploration (irrigation for agriculture); deforestation of caves surroundings (agriculture and pastures); climate change | Vulnerable (VU) | Not included | Limits of the Serra da Bodoquena National Park (part) |
| *Trichomycterus rubbioli* Bichuette & Rizzato, 2012 | Food restriction; physical change of the habitat; lowering of the upper phreatic | Deforestation of cave surroundings (agriculture; pastures and charcoal production); potential large scale mining projects; climate change | Vulnerable (VU) | Not included | Not included |
| *Trichomycterus* sp. "ramalho" | Lowering of the base-level stream; food restriction | Deforestation of cave surroundings (pastures and charcoal production); climate change | Not included | Not included | Not included |
| *Trichomycterus* sp. "iu iu" | Lowering of the base-level stream; food restriction | Deforestation for agriculture and pastures (small scale); climate change | Not included | Not included | Not included |
| *Ituglanis passensis* Fernández & Bichuette, 2002 | Lowering of the base-level stream; siltation of subterranean drainage; food restriction | Deforestation for agriculture and pastures (small scale); climate change | Vulnerable (VU) | Not included | Terra Ronca State Park |
| *Ituglanis bambui* Bichuette & Trajano, 2004 | Siltation of the upper vadose tributary; lowering of the upper vadose tributary; tourism | Unmanaged tourism; climate change | Critically Endangered (CR) | Not included | Terra Ronca State Park |
| *Ituglanis epikarsticus* Bichuette & Trajano, 2004 | Lowering of the epikarst (upper aquifer); physical change of the habitat; tourism | Climate change; unmanaged tourism | Vulnerable (VU) | Not included | Terra Ronca State Park |
| *Ituglanis ramiroi* Bichuette & Trajano, 2004 | Lowering of the upper vadose tributary; physical change of the habitat; tourism | Climate change; unmanaged tourism | Vulnerable (VU) | Not included | Terra Ronca State Park |

**TABLE 2** | (Continued)

| Species | Threats | Possible causes | IUCN Brazilian Red List (ICMBio/MMA, 2018) | IUCN Global Red List | Conservation Unit |
|---|---|---|---|---|---|
| *Ituglanis mambai* Bichuette & Trajano, 2008 | Food restriction; siltation of the subterranean drainage | Deforestation of surroundings (agriculture and pastures) | Endangered (EN) | Not included | Not included |
| *Ituglanis boticario* Rizzato & Bichuette, 2015 | Food restriction; siltation of the subterranean drainage | Deforestation of surroundings (agriculture and pastures) | Not included | Not included | Rio Vermelho Environmental Protection Area (APA) |
| *Ituglanis* sp. "terra ronca" | No data | No data | Not included | Not included | Terra Ronca State Park |
| *Ituglanis* sp. "posse" | Physical change of the habitat; lowering of the upper phreatic | Dams inside the cave for water exploration; climate change | Not included | Not included | Not included |
| *Ituglanis* sp. "canastra" | Food restriction; lowering of the base-level stream | Deforestation of surroundings; climate change | Not included | Not included | Limits of the Serra da Canastra National Park |
| *Glaphyropoma spinosum* Bichuette, de Pinna & Trajano, 2008 | Physical change of the habitat; tourism | Illegal gold panning ("garimpo"); unmanaged tourism | Vulnerable (VU) | Not included | Chapada Diamantina National Park |
| *Copionodon* sp. "igatu" | Physical change of the habitat; tourism | Illegal gold panning ("garimpo"); unmanaged tourism | Not included | Not included | Chapada Diamantina National Park |
| *Pimelodella kronei* (Miranda Ribeiro, 1907) | Pollution (domestic sewage and others); overcollecting; physical change of the habitat | Irregular land use; weak supervising; irregular visitation of the cave | Endangered (EN) | Deficient Data (DD) | Turístico do Alto Ribeira State Park |
| *Pimelodella spelaea* Trajano, Reis & Bichuette, 2007 | Lowering of the upper vadose tributary; physical change of the habitat; tourism | Climate change; unmanaged tourism | Endangered (EN) | Not included | Terra Ronca State Park |
| *Pimelodella* sp. "açungui" | Physical change of the habitat | Small Hydroelectric Power Station (SHPS) | Not included | Not included | Not included |
| *Rhamdia enfurnada* Bichuette & Trajano, 2005 | Food restriction; physical change of the habitat; lowering of the base-level stream | Deforestation of cave surroundings (agriculture; pastures and charcoal production); potential large scale mining projects; climate change | Not included | Not included | Not included |
| *Rhamdia* sp. "bodoquena" | Physical alteration of the habitat | Deforestation of cave surroundings; mining projects for cement production | Not included | Not included | Not included |
| *Rhamdiopsis krugi* Bockmann & Castro, 2010 | Siltation; food restriction; lowering of the aquifer; pollution (part of the aquifer); tourism (part of the caves) | Deforestation of caves surroundings; installation of artesian wells; use of pesticides for agriculture; unmanaged tourism | Vulnerable (VU) | Not included | Not included |

**TABLE 2** | (Continued)

| Species | Threats | Possible causes | IUCN Brazilian Red List (ICMBio/MMA, 2018) | IUCN Global Red List | Conservation Unit |
|---|---|---|---|---|---|
| *Rhamdiopsis* sp. "gonçalo" | Lowering of the aquifer; physical change of the habitat | Water withdrawal and installation of artesian wells for human consumption and agriculture use (small scale); old saltpeter exploration | Not included | Not included | Not included |
| *Rhamdiopsis* sp. "cordisburgo" | No data | No data | Not included | Not included | Not included |
| *Rhamdiopsis* sp. "ramalho", two populations/ morphotypes | Lowering of the upper phreatic; physical change of the habitat | Water withdrawal for human consumption and agriculture (small scale); dams inside the caves | Not included | Not included | Not included |
| *Rhamdiopsis* sp. "caatinga" | Lowering of the aquifer; physical change of the habitat | Water withdrawal for human consumption and agriculture use (small scale); dams inside the cave | Not included | Not included | Not included |
| *Phenacorhamdia* sp. "posse" | Physical change of the habitat; lowering of the upper phreatic | Dams inside the cave; water withdrawal; climate change | Not included | Not included | Not included |
| *Phreatobius cisternarum* Goeldi, 1905 | Habitat fragmentation; food restriction | Deforestation for agriculture and pasture activities | Least Concerned (LC) | Deficient Data (DD) | Not included |
| *Phreatobius dracunculus* Shibatta, Muriel-Cunha & de Pinna, 2007 | Habitat fragmentation; food restriction | Deforestation for agriculture and pasture activities; mining | Deficient Data (DD) | Not included | Not included |
| *Phreatobius sanguijuela* Fernández, Saucedo, Carvajal-Vallejos & Schaefer, 2007 | Habitat fragmentation; food restriction | Deforestation for agriculture and pasture activities | Not included | Critically Endangered (CR) | Not included |

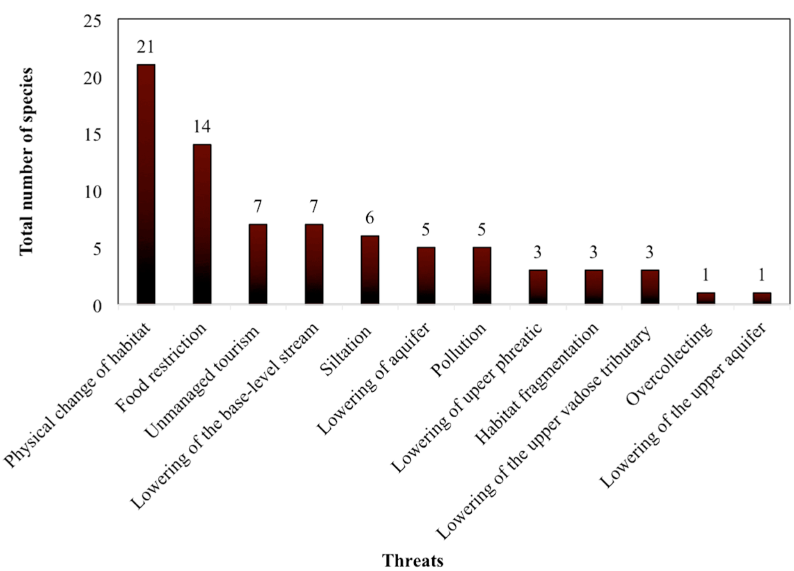


**FIGURE 6** | Total number of Brazilian subterranean fish species affected by each type of identified threat in the present study.

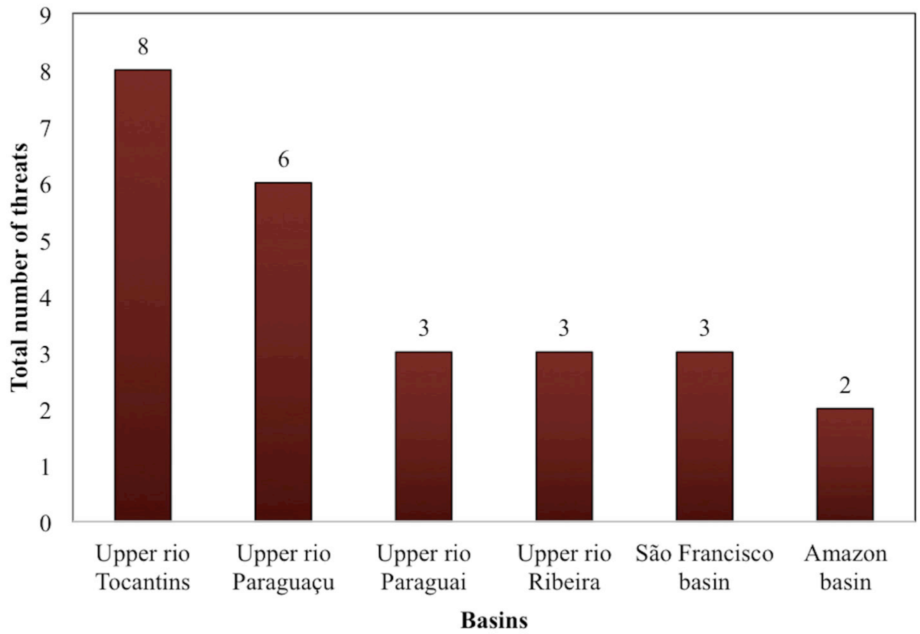


**FIGURE 7** | Total number of threats recorded for each basin.

## DISCUSSION

The distribution gaps observed to the sampling insufficiency in several Brazilian regions, which implies in a Wallacean shortfall, an ecological concept that refers to incomplete geographic distribution of most species (Hortal *et al*., 2015). One of the most challenging bottlenecks in planning the conservation of this ichthyofauna is the fact that, for most of the species, geographic distributions are poorly known. This issue is related to the specificity of habitats where these fishes occur, which can be interconnected in the same outcrop/landscape, on a small scale or in extensive aquifers, on a large scale. Projects considering the possibility of connectivity between these populations can address the question of effective proposal areas for conservation.

A high number of threats identified for a given species does not necessarily represent the worst-case scenario, since, for example, physical habitat changes alone (one of the threats identified for *Stygichthys typhlops*, one of the 11 species of São Francisco basin, in upper and middle portions) might mean that the entire habitat may be suppressed, due, for example, to mining, hydroelectrical impacts or water exploitation for large irrigation projects. In addition, when the number of threats to cave habitats per river basin is considered, the upper rio Tocantins stands out by the high number of identified threats (eight), but this is a consequence of its highly diverse subterranean ichthyofauna, including species that occur in unique and particular cave habitats (*e.g*., epikarst, upper vadose tributaries, base-level streams) (Gallão, Bichuette, 2018; Bichuette, Trajano, 2021; Bichuette, 2021). However, this idea must be considered with caution, since the upper rio Paraguaçu presented six threats and harbor only three subterranean species, one of the species, *Rhamdiopsis krugi* Bockmann & Castro, 2010, causes this bias, since the threats are several in the 12 caves where this species occurs.

Different threats can affect from biological, ecological, and evolutionary aspects of these populations. For example, *Stygichthys typhlops* is probably a relict, representing an unique lineage (Moreira *et al*., 2010) and the loss of phylogenetic information in a possible extinction of *Stygichthys typhlops* can be worst than for other subterranean fishes in Brazil, which belongs to genera widely distributed or relatively widely distributed. Anyway, any loss must be avoided.

Considering the high number of troglobitic fishes in Brazil and the absence of specific plans for the conservation of this particular ichthyofauna, the scenario is very concerning. Only the electric cavefish *Eigenmannia vicentespelaea* Triques, 1996 (VU), the trichomycterid *Trichomycterus itacarambiensis* Trajano & de Pinna, 1996 (CR) and the characiform *Stygichthys typhlops* (EN) are included in PANs ("Planos de Ação Nacional"), which preconize and suggest effective actions to protect the Brazilian biodiversity. *Eigenmannia vicentespelaea* in the "Plano de Ação Nacional para a Conservação do Patrimônio Espeleológico nas Áreas Cársticas da Bacia do Rio São Francisco"; *Trichomycterus itacarambiensis* in the "Plano de Ação Nacional para a Conservação das Espécies Ameaçadas de Extinção da Fauna Aquática da Bacia do Rio São Francisco" and *Stygichthys typhlops* in the "Plano de Ação Nacional para a Conservação das Espécies Ameaçadas de Extinção da Fauna Aquática da Bacia do Rio São Francisco" and the "Plano de Ação Nacional para a Conservação do Patrimônio Espeleológico nas Áreas Cársticas da Bacia do rio São Francisco" (Portaria MMA N° 358, September 30, 2009). None of these plans, however, was conceived with focus on one of these species,

Manjarrés-Hernández *et al.* (2021) discussed predictions of losses in the biodiversity of epigean freshwater fish species as consequences of the effects of climate change. They proposed a new and robust methodological approach to predict simple species distribution for future climatic scenarios (NOO3D, ModestR software). The authors predicted, from 16,825 freshwater fish species considered (1,464,232 occurrence records), the extinction of almost half the current freshwater fish species in the coming decades, with a pronounced decline in tropical regions and greater extinction likelihood for species with smaller body size and/or limited geographical ranges. These latter characteristics are the rule for subterranean fish populations in Brazil (Bichuette, Trajano, 2010; Bichuette, 2021), and studies focusing on the effects of climate changes particularly for the subterranean ichthyofauna are scarce, the need to provide information about it is urgent.

The limits of the area proposed for the protection of a given subterranean fish species, in addition to encompassing the habitat itself, must also include the micro basins and associated aquifers in which the habitat is included. This is due to the important fact that a large part of the trophic resources present in the subterranean environment is of allochthonous origin, and broader landscape impacts exert strong influences on the subterranean biota. The possibility of including undescribed species in Red Lists should also be considered, at least in the case of undescribed species with already identified, unequivocal diagnoses, and that have testimony specimens deposited in accredited scientific collections, as discussed by Gallão, Bichuette (2012).

Trajano (1997, 2008a) addressed the concern about threats identified for *Pimelodella kronei* and remarked the necessity of protection of this species. Gallão, Bichuette (2012) reinforced the importance of protection of the Brazilian subterranean ichthyofauna through the use of the IUCN Red List for effective actions. These alarming concerns, however, remain, exacerbated by the increase in the number of undescribed species

(Linnean shortfall – a concept that considers most of the species on Earth have not been described and/or cataloged) (Hortal *et al*., 2015) and not evaluated in the regional, national and global lists of threatened fauna, in addition to the recent increase in the number of threats for most Brazilian landscapes and their aquatic ecosystems, including the subterranean ones. Allied to this fact, we have the current dismantling and attacks to the environmental policies in Brazil, with a significant increase in fires, deforestation, and the transfer of areas to the production sector and other activities (Thomaz *et al*., 2019).

A possible solution for proposing and taking actions towards the protection of such particular ecosystems and their communities, not limited to the evaluation of the species themselves, is a recent proposal by the IUCN of developing categories and criteria for a Red List of Ecosystems (RLE) aimed to ecosystem risk assessment at multiple scales (Keith *et al*., 2015). According to this proposal, the RLE would include eight categories of risk for each ecosystem based on five quantitative criteria designed to evaluate risk symptoms in terrestrial, subterranean, freshwater, and marine ecosystems (Keith *et al*., 2013). Subterranean ecosystems are being considered in the development of RLE criteria, which has 2025 as a target date to achieve global coverage of ecosystems. The authors hope that the Brazilian subterranean fishes resist and survive until that aim is achieved.

Finally, we reinforce the importance of long-term monitoring and functional approach projects, still scarce, in conservation program proposals, allied to education projects aiming to develop public awareness.

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**Maria Elina Bichuette:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing-original draf.

**Jonas Eduardo Gallão:** Writing-original draf, Writing-review and editing.

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