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Amphibian Illegal Pet Trade and a Possible New Case of an Invasive Exotic Species in Brazil

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Abstract.—**Illegal wildlife trade is historical and has not been contained. With the popularization of the internet and social media globally, we hypothesized that illegal trade would be facilitated and predict that trade will increase. To test the hypothesis, we surveyed amphibian illegal trade on Facebook groups in the last 5 y between 2015 and 2020 in Brazil and compared our results to previous studies conducted a decade ago. We also tested whether some of the illegally traded amphibians were carrying the chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), by means of qPCR analyses. We also looked for information about new exotic species in Brazil. We found that the online illegal trade was indeed increasing in Brazil, becoming at least six times higher than previously reported. We did not find *Bd* in traded amphibians whereas we found an individual frog of the non-native Asian microhylid *Kaloula pulchra* in the wild in northeastern Brazil. Retrieving information about the illegal trade on the internet was easy; therefore, we advocate for a rapid and efficient action by law enforcement agents to stop or restrain the current amphibian illegal trade. Although we did not find *Bd* in the sampled amphibians, the sample size was small (n = 45) and thus it is still possible that the trade is contributing to the spread of the pathogens.**

Key Words.—amphibian conservation; Anura; Caudata; international trade; online wildlife market

Introduction

Illegal wildlife trade constitutes one of the major illegal economic activities worldwide (Berber-Meyer 2010; South and Wyatt 2011) that has various negative impacts on native species and is a serious wildlife conservation problem (Salati et al. 2007). Vertebrates are most affected, and amphibians are the most threatened vertebrate group (Hoffmann et al. 2010). Besides removing individuals from their natural habitats, one of the consequences of the international illegal trade of amphibians was the widespread introduction of exotic species (e.g., North American Bullfrogs, *Aquarana catesbeiana*). After establishing resident populations across large areas (Both et al. 2011), bullfrogs compete with native amphibians for resources such as food (Shine 2010), acoustic niche (Forti et al. 2017), prey upon local fauna (Snow and Witmer 2010) and spread of lethal pathogens (Schloegel et al. 2009; O'Hanlon et al. 2018; Brunner et al. 2019). The number of exotic amphibian species invading new sites is increasing exponentially (Forti et al. 2017), therefore stopping worldwide illegal trade in organisms is an important conservation goal (Toledo et al. 2012).

Legislation to limit illegal trade include CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora), which regulates the global animal (and plant) trade, tracking records of the importations and exportations of the most vulnerable fauna (Doukakis et al. 2012). Also, diseases are internationally monitored; in the case of amphibians, the most important pathogens, *Batrachochytrium dendrobatidis* (*Bd*), *B. salamandrivorans* (*Bsal*), and *Ranavirus* spp. (*Rv*) are globally tracked by the World Organization for Animal Health, the OIE (Schloegel et al. 2010). In addition to these international regulations, every country has its protection system and laws.

In Brazil, an ordinance published in 1998 established rules for the importation of live animals, including exotic and native species (Brasil 1998). As a result, in that year and in the subsequent decade (1998–2009), 20 amphibian species were seized by the police or other governmental organizations, and 13 native species were found to be commercialized in foreign websites (Pistoni and Toledo 2010). Additionally, from 2006 to 2012, five amphibian species were found to be illegally traded via Orkut (Magalhães and São–Pedro 2012), the most popular social media in Brazil at that time. Three of the five species listed were anurans (Pistoni and Toledo 2010; Magalhães and São-Pedro 2012) and two were salamanders. A recent study (Fonseca et al. 2019) added one more anuran species, the Indian Bullfrog (*Rhinella*

cf. *margaritifera*; published as *Hoplobatrachus tigerinus*), in the Brazilian pet trade records and the CITES online trade database lists other individual dendrobatids, such as the Rio Madeira Poison Frog (*Adelphobates quinquevittatus*), the Yellow-banded Poison Frog (*Dendrobates leucomelas*), and other poison frogs (*Dendrobates* spp.) that were confiscated between 1997 and 2011 (United Nations Environment Programme, World Conservation Monitoring Centre. 2020. Trade database. CITES. Available from https://trade.cites.org/en/cites_trade [Accessed 13 July 2020]). It is possible that many other species are being illegally traded via the internet and have not been found by law enforcement agents or reported in past studies.

A decade after those studies focusing on the illegal pet trade (Pistoni and Toledo 2010; Magalhães and São-Pedro 2012), the percentage of the Brazilian population with access to the internet grew from < 1.5% in 1998 to 61% in 2016 (Roser, M., H. Ritchie, and E. Ortiz–Ospina. 2015. Internet. Global Change Data Lab. Available from https://ourworldindata.org/internet [Accessed 13 July 2020]). Today, more than 70% of Brazilians have access to the world wide web (Chapchap, G. 2020. Mais usuários de internet no Brasil: mais oportunidades para o e–commerce. E-Commerce Brasil. Available from https://www.ecommercebrasil.com.br/artigos/mais-usuarios-internet-mais-oportunidades/ [Accessed 27 June 2020]). Also, the preferred social media in Brazil has changed from Orkut to Facebook, with over 127 million users monthly (Kaufman 2019). With such an increase in internet popularity and users, we wanted to evaluate the hypothesis that amphibian illegal e–commerce has grown from the Orkut to the Facebook era. We predicted that illegal trade in amphibians in Brazil is significantly higher now than in the past. Additionally, we tested some individuals of the current Brazilian illegal pet trade for the presence of *Batrachochytrium dendrobatidis*, a major cause of amphibian declines worldwide (Scheele et al. 2019) and in Brazil (Carvalho et al. 2017). We also report a new case of an invasive amphibian in northeast Brazil, likely as a consequence of the illegal pet trade.

Materials and Methods

We searched for Brazilian Facebook posts of amphibians using Portuguese keywords such as *anfíbio* (amphibian), *anfíbios* (amphibians), *sapo* (toad), rã (frog), *perereca* (treefrog), *salamandra* (salamander), and *venda* (sale). Two groups with amphibian sales were found between October 2019 and June 2020 and we gathered data from such online sale advertisements from January 2015 to June 2020. In many cases, we were able to contact the dealers to acquire more information, such as price, quantity, and origin of individuals. If the number of individuals were not provided, we estimated the number of individuals in the published photographs. If there were no photographs, or any additional information, we considered there was a minimum of one or two individuals for sale, depending on if the person referred to the animal with singular or plural words, respectively. We converted the sales price to U.S. dollars, using the exchange rate from 30 April 2020 (1 USD = 5.33 BRL) and rounded values to integer numbers. Most posts contained a photograph or a video of the animal. Thus, we used these, combined with additional information provided by dealers/owners, for species identification. A species was considered native if it has natural distribution in Brazil, or alien/exotic if it occurs naturally only outside of Brazil. We also provide the threat category based on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (IUCN 2020), and the CITES appendix list in which it is included (United Nations Environment Programme - World Conservation Monitoring Centre. 2020. *op. cit.*).

Besides collecting data on the internet, we had access to animals that were being sold in physical stores, were in possession of pet owners, or that were seized. We swabbed 36 of the 43 Axolotls (*Ambystoma mexicanum*) apprehended in October 2019 by the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) in Brasília of the Federal District, and we analyzed them for Bd infection. Besides these animals, we also swabbed nine African Clawed Frogs (*Xenopus laevis*): two were from a pet owner who sent us the animals in April 2017, three were swabbed directly in a fish store (where they were to be sold illegally) in Campinas, São Paulo, Brazil, in February 2011, and four were seized by the police, in Diadema, São Paulo, in November 2020. We skin-swabbed these specimens following the non-invasive protocol for *Bd* collection (Hyatt et al. 2007). We used a new pair of gloves before handling each amphibian to prevent cross-contamination between animals in case *Bd* was present. Swabs were individually stored in cryotubes and kept at -20° C. In the laboratory at Unicamp, we extracted the DNA from each swab using PrepMan® Ultra Sample Preparation Reagent (Thermo Fisher Scientific, Warrington, UK) and we performed a qPCR TaqMan assay for *Bd* detection and quantification (Boyle et al. 2004; Lambertini et al. 2013). We considered positive (Bd^+) those samples that amplified at least one zoospore genomic equivalent (g.e.) (Hyatt et al. 2007). Besides these, in January 2021, a Russian animal dealer was arrested at the São Paulo / Guarulhos international airport (GRU) with a large shipment that included, among others, amphibians endemic to Brazil that were about to be moved to Saint Petersburg (pers. obs.). We listed these specimens, but

had no access to them and could not test for *Bd*. Also, in June 2020, an exotic frog was found by residents feeding on a bee colony in the municipality of Campo Formoso, state of Bahia, northeast Brazil. It was photographed but not collected.

Results

On Facebook groups, sellers were mainly from the states of Rio de Janeiro and São Paulo, but we also tracked pet owners from the states of Minas Gerais and Pernambuco. We listed 69 sellers/pet owners and 125 species advertisements of at least 31 amphibian species (Appendix Table). We found that the advertisements occurred every year, and overall, 54% of the individuals being illegally traded were of exotic species (13 species), versus 46% native species (18 confirmed species, seven unknown; Fig. 1, Appendix Table). We could not gather much information about the origin of the specimens being sold. The only exotic individuals for which we had some information were from the Marbled Newt (*Triturus marmoratus*), whose seller informed us that they were directly imported from Spain. Native species were mostly directly removed from the wild.

We did not detect the presence of *Bd* in any of the *A. mexicanum* or *X. laevis* individuals sampled. Among the seized animals at GRU airport in 2021, there were 87 individuals of six anuran species, all endemic to the Southern Atlantic Forest, and only two of them had already been reported in the illegal trade (Table 1). We identified the record of the exotic frog in Campo Formoso as the Painted Bullfrog (*Kaloula pulchra*; Anura, Microhylidae, Microhylinae) based on good-quality photographs.

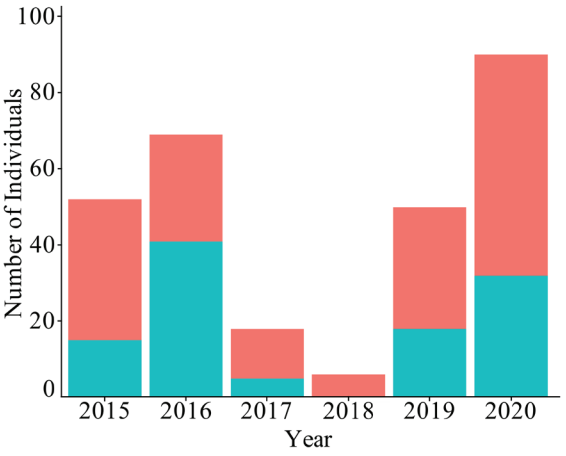

Figure 1. Number of individuals from exotic (coral color) and native amphibian species (turquoise color) found in online illegal pet trade per year in Brazil.

Discussion

Comparing our data (2015–2020) with the previous information gathered also from social media and in a comparable timeframe (2006–2012: Magalhães and São–Pedro 2012), we were able to detect a six-fold increase in the number of species being sold in the online market (from five to at least 31 species). That is alarming as it may indicate this market is not only continuing but increasing, and more effort will be necessary to combat such illegal activity. Finding these sales groups and contacting dealers was not difficult, so we believe that law enforcement agents should use this available information and act rapidly against such adverse practices.

Table 1. Amphibian species seized (n = number) at the São Paulo/Guarulhos international airport (GRU), Brazil, 20 January 2021. Species origin, presence in Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) appendices, and International Union for Conservation of Nature (IUCN) conservation status; LC = Least Concern. An en-dash (–) stands for information not available. An asterisk (*) indicates those species that have already been reported in the illegal amphibian trade in Brazil.

Scientific name	Common name	n	Species origin	CITES	IUCN
Anura					
Bufonidae					
*Rhinella icterica**	Cururu Toad	16	Native	–	LC
R. ornata	–	10	Native	–	LC
Microhylidae					
Chiasmocleis leucosticta	Humming Frog	12	Native	–	LC
Odontophrynidae					
Macrogenioglottus alipioi	Wanderer Frog	6	Native	–	LC
*Proceratophrys boiei**	Smooth Horned Frog	37	Native	–	LC
Phyllomedusidae					
Phyllomedusa distincta	Leaf Frog	6	Native	–	LC

Some species seem to be sporadically traded, but others are historically traded. For example, dendrobatids (traded as poison frogs), phyllomedusids (leaf or monkey frogs), fire-bellied toads (*Bombina* spp.), pacman frogs (*Ceratophrys* spp.), *Aquarana catesbeiana*, cane or common toads (*Rhinella* spp.), milk frogs (*Trachycephalus* spp.), *Xenopus laevis*, *Ambystoma mexicanum*, and Fire-bellied Newts (*Cynops orientalis*) have been traded over decades (Pistoni and Toledo 2010; Magalhães and São–Pedro 2012; this study). Most of these species were also reported among the most common species in YouTube videos of captive amphibians (Measey et al. 2019). Hence, some of the species might not be able to be caught in the field anymore but are being breed in captivity. On the other hand, a recent online market of the so-called Brazilian Horned Pacman (*Ceratophrys aurita*) on U.S. websites indicates that at least some individuals were illegally removed from the wild to be bred and internationally traded. Similarly, most (if not all) native species of brachycephalids, most hylids, leptodactylids, and odontophrynids, are most likely being collected in the wild.

As all sampled individuals were free of *Bd*, it is possible that *Bd* transmission in the Brazilian pet trade is low. Our sample size is still small to draw this conclusion, however, and pet amphibians found in the future must be screened not only for *Bd*, but also for *Bsal* and *Rv*. The latter two pathogens have also been responsible for amphibian mortalities (Martel et al. 2013; Duffus et al. 2015). So far, *Bsal* has not been reported from Brazil, and its arrival must be avoided. On the other hand, *Rv*, previously detected in frogs in the legal commercial trade (Brunner et al. 2019), has been recently found in the Atlantic Forest (Ruggeri et al. 2019), and already been associated with local anuran die-offs, both in frog farms (Mazzoni et al. 2009) and in the wild (Ruggeri et al. 2019).

The single wild caught *Kaloula pulchra* we confirmed represents a new record of an introduced amphibian in Brazil (Forti et al. 2017). We do not know if there is an established population in Campo Formoso. This species is commonly collected for the pet trade (AmphibiaWeb. 2009. *Kaloula pulchra*. AmphibiaWeb. Available from http://amphibiaweb.org/species/2157 [Accessed 27 June 2020]), and is originally from South Asia (Frost, D.R. 2020. Amphibian Species of the World: an Online Reference. Self-published. Available from: https://amphibiansoftheworld.amnh.org/index.php [Accessed 27 June 2020]). It may have been introduced in Guam (Christy et al. 2007), Borneo, Sulawesi, and in several islands of the Philippines (Frost, D.R. 2020. *op. cit.*). To our knowledge, there are no other records of a successful invasion of this species in South America; however, similar to our finding, another adult individual was collected in the wild in Argentina in 1986 (Faivovich and Elias-Costa 2020), but a population did not establish. Both records (in Brazil and Argentina) could represent single individual escapes of introduced specimens that were not able to establish invasive populations (Falaschi et al. 2020). Also, these records indicate that this species likely has been sold in South America for decades but was not detected in any of the previous surveys, including our study using data from Facebook groups. Accordingly, the discovery of a single non-native frog specimen in Campo Formoso may not represent a recent introduction, as a considerable time lag may have existed between the original introduction and the recognition by the scientific community of that invasion (Toledo and Measey 2018). Thus, we advocate for future field samplings in the region to evaluate the possibility of an established population.

Indeed, the establishment risk of exotic amphibian species is high (Kopecký et al. 2016; Stringham and Lockwood 2018). One of the most successful anuran invader species is the *Aquarana catesbeiana*, with feral populations found worldwide (Jorgewich-Cohen et al. 2020). This species represents an increasing conservation problem, not only due to their potential to adapt and invade new habitats (Johovic et al. 2020) but also because this species is spreading lethal pathogens (as *Bd* and Ranavirus) globally (O'Hanlon et al. 2018; Brunner et al. 2019). Similarly, *Xenopus laevis* is a carrier of *Ranavirus* spp. (Robert et al. 2007; Soto-Azat et al. 2016) and has a great invasion potential (Measey et al. 2012). Both species have been detected in the illegal pet trade in Brazil and constitute a threat for the Brazilian amphibian fauna. Thus, even though there is no report of invasive caudates (salamanders and newts) in Brazil, the highly pathogenic *Bsal* is known to infect many species in this order and could represent a threat for Brazilian salamanders of the genus *Bolitoglossa* if the imported pets are infected (see Stegen et al. 2017).

Besides the ecological impact caused by the establishment of exotic species, there are also social-economic consequences (Measey et al. 2016). Invasive populations of Lesser Antillean Whistling Frog (*Eleutherodactylus johnstonei*) have been found in Caribbean islands, Europe, and in South America, including Brazil (Ernest et al. 2012; Toledo and Measey 2018; Leonhardt et al. 2019). Besides direct environmental impacts, this species can transmit parasites to both native amphibians and humans (Linzey et al. 1998; Measey et al. 2016) and may cause depreciation of real estate due to the noise pollution caused by the loud calls of this amphibian (Forti et al. 2017; Toledo and Measey 2018). As we observed, the illegal pet trade is increasing, novel exotic species are at imminent risk of establishing new invasive populations, and lethal diseases are likely to be moving with these

exotic amphibians. Hence, the illegal pet trade in Brazil is a serious conservation problem that should be promptly addressed and considered in national action plans.

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Appendix Table. Species illegally commercialized on Facebook between January 2015 and June 2020. Species, number of individuals being sold (n), mean price (range in parentheses when there were multiple prices) converted to US dollars, year of advertisement, species origin, and presence in Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) appendices, and International Union for Conservation of Nature (IUCN) conservation status. An en-dash (–) stands for information not available. The asterisk (*) = possible that this refers to Orange-legged Leaf Frog (*Pithecopus hypochondrialis*), long confused with *P. azureus* (Bruschi et al. 2013).

Scientific name	Common name	n	Price (USD)	Advertisement year	Species origin	CITES	IUCN
Anura							
Bombinatoridae							
Bombina orientalis	Fire-bellied Toad	2	41	2015	Exotic	–	LC
B. orientalis	Fire-bellied Toad	1	–	2019	Exotic	–	LC
B. orientalis	Fire-bellied Toad	1	–	2020	Exotic	–	LC
Brachycephalidae			–				
Brachycephalus rotenbergae	–	3	–	2019	Native	–	LC
Bufonidae			–				
Rhinella icterica	Cururu Toad	5	–	2020	Native	–	LC
R. marina	Cane Toad	1	–	2015	Native	–	LC
R. marina	Cane Toad	1	–	2016	Native	–	LC
R. marina	Cane Toad	2	–	2019	Native	–	LC
R. marina	Cane Toad	1	11	2020	Native	–	LC
Rhinella sp.	Cururu toad	3	–	2016	Native	–	–
Ceratophryidae							
Ceratophrys ornata	Pacman	4	–	2015	Native	–	NT
C. ornata	Pacman	22	55	2016	Native	–	NT
C. ornata	Pacman	1	93	2017	Native	–	NT
C. ornata	Pacman	2	60 (46-74)	2020	Native	–	NT
Lepidobatrachus laevis	Budgett's Frog	1	–	2017	Exotic	–	LC
Dendrobatidae							
Adelphobates galactonotus	Poison Frog	2	55	2016	Native	II	LC
A. galactonotus	Poison Frog	5	104 (69-138)	2019	Native	II	LC
A. galactonotus	Poison Frog	4	68 (64-74)	2020	Native	II	LC
Dendrobates tinctorius	Poison Frog	7	104 (69-138)	2019	Native	II	LC
Ranitomeya sp.	Poison Frog	6	55	2020	Native	II	–
Hylidae							
Aplastodiscus arildae	Treefrog	10	9	2016	Native	–	LC
A. arildae	Treefrog	2	18	2020	Native	–	LC
Aplastodiscus sp.	Treefrog	1	6	2020	Native	–	–
Boana albomarginata	Treefrog	1	–	2016	Native	–	LC
B. albomarginata	Treefrog	1	28	2020	Native	–	LC
Dendropsophus microps	Treefrog	1	24	2020	Native	–	LC
D. werneri	Treefrog	1	24	2020	Native	–	LC
Scinax fuscovarius	Snouted Treefrog	1	–	2020	Native	–	LC
Scinax sp.	Snouted Treefrog	1	–	2015	Native	–	LC

Appendix Table (continued).

Scientific name	Common name	n	Price (USD)	Advertisement year	Species origin	CITES	IUCN
Trachycephalus sp.	Milk frog	2	40 (33-46)	2020	Native	–	LC
T. typhonius	Milk Frog	1	–	2016	Native	–	LC
Leptodactylidae							
Physalaemus cuvieri	Barker Frog	1	–	2015	Native	–	LC
P. cuvieri	Barker Frog	1	28	2020	Native	–	LC
Odontophrynidae							
Proceratophrys boiei	Smooth Horned Frog	1	–	2020	Native	–	LC
Phyllomedusidae							
Agalychnis callidryas	Red-eyed Treefrog	9	74	2019	Exotic	II	LC
Callimedusa tomopterna	Tiger-striped Leaf Frog	1	37	2020	Native	–	LC
Phyllomedusa tetraploidea	Leaf Frog	2	74	2015	Native	–	LC
*Pithecopus azureus**	Leaf Frog	1	–	2015	Native	–	DD
*P. azureus**	Leaf Frog	1	–	2016	Native	–	DD
*P. azureus**	Leaf Frog	1	–	2019	Native	–	DD
P. hypochondrialis	Leaf Frog	2	–	2020	Native	–	LC
Pipidae							
Xenopus laevis	African Clawed Frog	10	3	2015	Exotic	–	LC
X. laevis	African Clawed Frog	6	–	2016	Exotic	–	LC
X. laevis	African Clawed Frog	4	12 (9-14)	2017	Exotic	–	LC
X. laevis	African Clawed Frog	1	13	2018	Exotic	–	LC
X. laevis	African Clawed Frog	1	9	2019	Exotic	–	LC
X. laevis	African Clawed Frog	49	22 (9-44)	2020	Exotic	–	LC
Ranidae							
Aquarana catesbeiana	North American Bullfrog	13	4 (3-5)	2015	Exotic	–	LC
A. catesbeiana	North American Bullfrog	8	9	2016	Exotic	–	LC
A. catesbeiana	North American Bullfrog	7	–	2017	Exotic	–	LC
A. catesbeiana	North American Bullfrog	4	–	2018	Exotic	–	LC
A. catesbeiana	North American Bullfrog	4	28	2019	Exotic	–	LC
A. catesbeiana	North American Bullfrog	1	18	2020	Exotic	–	LC
Not identified							
–	Tadpoles	3	–	2015	Native	–	–
Caudata							
Ambystomatidae							
Ambystoma mexicanum	Axolotl	9	–	2015	Exotic	II	CR
A. mexicanum	Axolotl	10	28 (22-33)	2019	Exotic	II	CR
A. mexicanum	Axolotl	1	–	2020	Exotic	II	CR
A. opacum	Marbled Salamander	2	552	2019	Exotic	–	LC
A. tigrinum	Eastern Tiger Salamander	1	368	2020	Exotic	–	LC
Plethodontidae							
Bolitoglossa sp.	Lungless Salamander	4	–	2017	Native	–	DD

Appendix Table (continued).

Scientific name	Common name	n	Price (USD)	Advertisement year	Species origin	CITES	IUCN
Salamandridae							
Cynops orientalis	Fire-bellied Newt	1	–	2015	Exotic	–	LC
C. orientalis	Fire-bellied Newt	1	–	2017	Exotic	–	LC
Pleurodeles waltl	Sharp-ribbed Salamander	14	28	2016	Exotic	–	NT
P. waltl	Sharp-ribbed Salamander	2	33	2019	Exotic	–	NT
Salamandra salamandra	Spotted Salamander	1	–	2018	Exotic	–	LC
Triturus cristatus	Crested Newt	3	52	2019	Exotic	–	LC
T. cristatus	Crested Newt	3	52	2020	Exotic	–	LC
T. marmoratus	Marbled Newt	2	221	2020	Exotic	–	LC

Citations of the appendix table:

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