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Risks associated with introduction of poeciliids for control of mosquito larvae: first record of the non-native *Gambusia holbrooki* in Argentina

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This study confirms the presence of two species of the non-native mosquitofish *Gambusia* in Argentina. The risks that they represent to native biota, their potential dispersal in the region, and their effectiveness in mosquito larvae control are discussed.

Key words: *coI*; cryptic species; Cyprinidontiformes; exotic species; *Gambusia*; mosquitofish.

The mosquitofishes *Gambusia holbrooki* Girard 1859 and *Gambusia affinis* (Baird & Girard 1853) are small fishes of the family Poeciliidae native to freshwater basins of the east coast of the U.S.A. Over the past century, they have been introduced in many areas worldwide owing to their presumed ability to control mosquito larvae (García-Berthou *et al.*, 2005). The characteristic broad diet and physiological tolerance of species of *Gambusia* Poey 1854, their rapid population growth, high genetic variability, aggressive behaviour, and dispersal tendencies (Stockwell & Henkanaththegedara, 2011), make them capable of adapting to and becoming established in a wide variety of environments (Vidal *et al.*, 2010). *Gambusia affinis* has been included in a list of 100 invasive non-native species with the greatest cosmopolitan effect (Lowe *et al.*, 2000).

Gambusia affinis and *G. holbrooki* are markedly sexually dimorphic, with females significantly larger than males and males possessing an anal fin modified as

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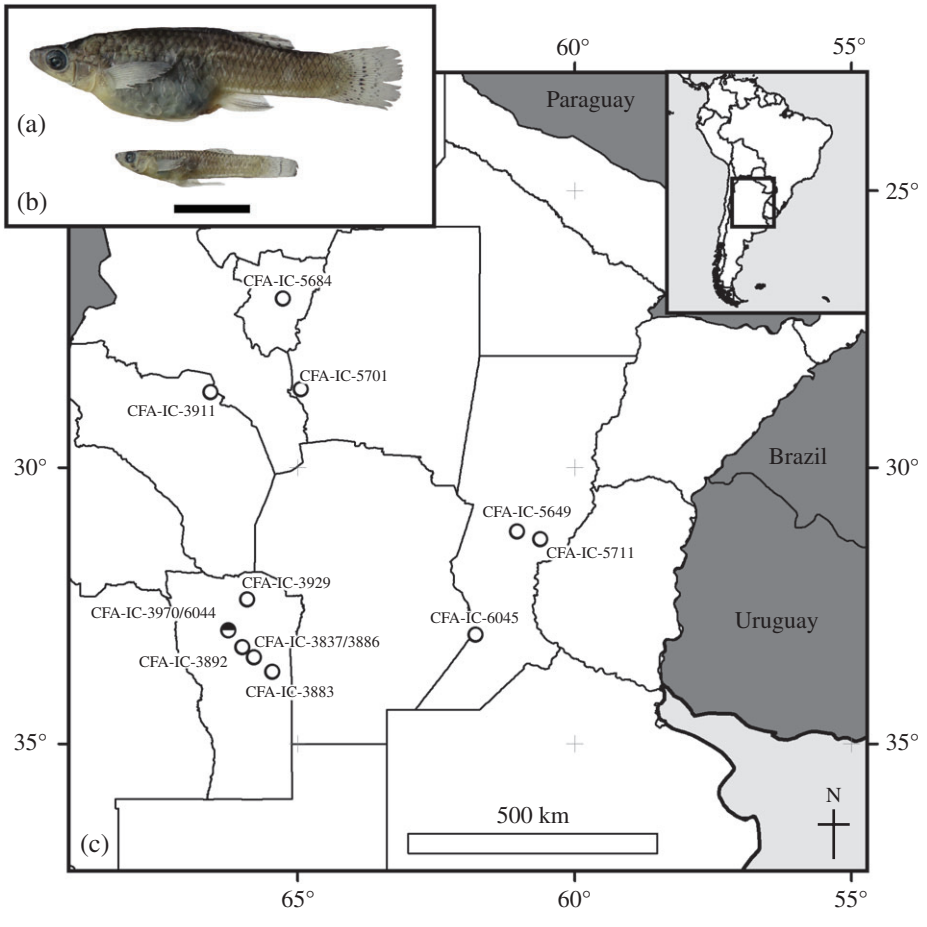

FIG. 1. (a) Female and (b) male *Gambusia holbrooki* collected at River Quinto, San Luis. Scale bar = 1 cm, (c) The sampling localities for *G. holbrooki* (○) and sympatric occurrence of *G. affinis* and *G. holbrooki* (◓) in Argentina with collection codes (for more details see Appendix S1).

a gonopodium [Fig. 1(a)]. The species are similar morphologically and ecologically. Their primary diagnostic character is the morphology of the gonopodium, making females cryptic and a challenge to discriminate (Rauchenberger, 1989; Walters & Freeman, 2000). Identification of the species is a global issue, both in their native territory and in areas into which they have been introduced (Walters & Freeman, 2000). Representatives of *Gambusia* in Australia were not identified as *G. holbrooki* until the 1980s (Lloyd & Tomasov, 1985), with almost every earlier reference to Australian specimens given as *G. affinis*. In Europe, documents indicate that the introduction of both species took place in 1920, but in a genetic analysis of >400 samples in six European countries, Vidal *et al.* (2010) identified only *G. holbrooki*. In Argentina, specimens have always been designated as *G. affinis* (as in Cardoso *et al.*, 2015), but it is likely that several citations of *G. affinis* in the country are *G. holbrooki*.

Using the presence or absence of a gonopodium, samples were separated by sex and identified following Rauchenberger's (1989) criteria (Fig. 2). The rays on the

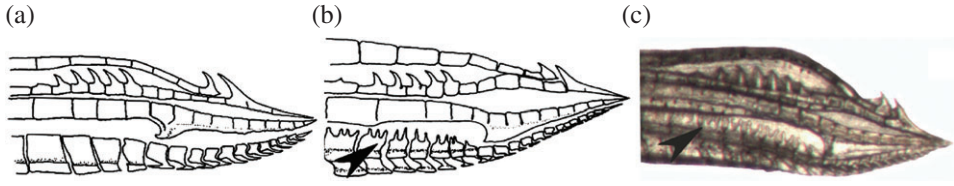

FIG. 2. (a) Gonopodium of *Gambusia affinis* and (b) *Gambusia holbrooki*, modified from Rauchenberger (1989). (c) Detail of the gonopodium of *G. holbrooki* from the River Quinto in San Luis Province. ⮟, the characteristic spines of *G. holbrooki* on the third ray.

dorsal and anal fins were counted in females. Nevertheless, as Walters & Freeman (2000) suggested, these characters overlap between species (Appendix S1, Supporting Information). To identify cryptic species reliably, molecular methods, such as DNA barcoding using the mitochondrial cytochrome oxidase c subunit I marker (*coI*, *c.* 650 bp), are recommended (Hebert *et al.*, 2003). Eight specimens were sequenced for the *coI* gene and the sequences deposited in GenBank under accession numbers KX512843-50. The sequences were searched by basic local-alignment search tool (BLAST)n against GenBank and in the Barcode Index Number (BIN) system in the Barcode of Life Data (BOLD) identification engine (Ratnasingham & Hebert, 2013). Five sequences were genetically identical and had 100% identity with Genbank accession number JN026703, corresponding to a specimen from the U.S.A. identified as *G. affinis*, and with BIN ACC2756, which also corresponds to this species. The three remaining sequences showed 99% similarity with GenBank accession numbers HQ937054/JN026706/JQ979157/JX049126, corresponding to *G. holbrooki* from the U.S.A. and Turkey, and 100% similarity with BIN ACC2757, which corresponds to *G. holbrooki.* Accurate identification to species is important for the monitoring of cryptic species and for the detection of introductions, and DNA barcoding has proven to be one of the most promising methods for this purpose.

To date, *G. affinis* is the only species of *Gambusia* reported in Argentina. In 1943, several populations were introduced for malaria control (Ringuelet *et al.*, 1967). Although it is believed that these fishes were imported from U.S.A. and Chile (acquired from the Departamento de Pesca y Caza de Chile where they were introduced in 1937), there has been no definitive information about the origin of the specimens or observations on the effects they have generated. The present study confirms the presence of two species of *Gambusia* in Argentina. Interestingly, only one occurrence locality was found for *G. affinis* in the Nogoli River, where the two species are sympatric [Fig.1 (b)]. The list of examined material and a brief description of *G. holbrooki* collected in Argentina can be found in Appendix S1.

The effect of *Gambusia* spp. in Argentina has not been studied, nor has the success of its invasion in the country. Several studies, however, on the effect of the Poeciliidae on native biota in other areas of the world have been published. Stockwell & Henkanaththegedara (2011) reviewed data of the extent to which these introduced species have caused decline in, or even extirpated native fish species through competition for resources, predation on eggs, larvae, and adults, transmission of parasites and disease, aggressive behaviour causing injury and mortality, and hybridization. It is

well documented that species of *Gambusia* can negatively affect invertebrates, plants, and amphibians (Pyke, 2008; Stockwell & Henkanaththegedara, 2011).

In field sampling campaigns, the observed abundance of *Gambusia* in some environments was greater than that of native species (Y. P. Cardoso pers. obs.). Even without empirical data on the proliferation of *Gambusia* in the region, the observations of this study suggest that their distribution area has increased in recent years, typical of any successful invasion (Sakai *et al.*, 2001). Six previously unreported locations were found in Argentina, beyond the usual distribution of species of *Gambusia*. In these locations, 11 endemic species of Cyprinodontiformes with similar ecological adaptations to those of *Gambusia* were present and could be severely affected. The introduction of invasive species can generate extinction of other species, and has become one of the most common threats to endemic biodiversity. The current findings call attention to the effect that species of *Gambusia* may have on southern South America. Most of the sampled localities occur in endorheic river systems. These closed basins represent a natural restriction to dispersal of fishes; nevertheless, *Gambusia* was also found in the La Plata River basin. This catchment, with an area of 3·2 million km^2, extends into five countries: Brazil, Paraguay, Bolivia, Uruguay, and Argentina. Owing to their extreme flexibility in adapting to a wide variety of environments, the species of *Gambusia* reported here show potential for widespread invasion of southern South America.

Thousands of specimens of *Gambusia* have been introduced into South America in the past 5 years to control epidemic diseases transmitted by *Aedes* mosquitoes, including Dengue, Chikungunya, and Zika. Controlling mosquito populations is the primary strategy in combating vector-transmitted disease and the introduction of fishes as biological controls appears to have been successful *v*. alternatives such as the use of insecticides and herbicides (Gozlan *et al.*, 2010). Historically, public-health agencies have used poeciliids for control of mosquitoes. Nonetheless, the effects on the recipient ecosystems must be considered, as several introductions have resulted in biological invasions, a complex phenomenon with highly negative implications (Simberloff *et al.*, 2013). There is also uncertainty over the level of biological control that will be achieved and at what time scale, leading to considerable disagreement over the concept of bio-control (Gozlan *et al.*, 2010). The diet of *Gambusia* is diverse, including larval and adult crustaceans, insects, molluscs, annelids, algae, aquatic plants, detritus, and juveniles of other species of fishes (Rodríguez-Jiménez, 1989; García-Berthou, 1999; Rehage *et al.*, 2005; Nieva *et al.*, 2010). This has raised doubt as to its effectiveness in mosquito control (Pyke, 2008). Breeding *Aede*s mosquitoes are rarely found in the wilderness environments into which *Gambusia* is often released. These mosquitoes have peri-domiciliary habits (Healy *et al.*, 2014), and the diseases they transmit are predominantly urban. It is strongly recommended that public-health authorities control mosquito-borne disease *via* environmentally sound strategies, including development of vaccines as well as community education to reduce the peri-domestic mosquito populations (Healy *et al.*, 2014; Azevedo-Santos *et al.*, 2016).

Currently, public agencies expend considerable funds on the introduction of exotic species, with limited knowledge of their taxonomy, their processes of invasion, and the ecological risk involved, or of their efficacy in biological control (El-Sabaawi *et al.*, 2016; Servick, 2016). Cost–benefit analyses associated with the introduction of bio-controls are difficult to conduct, since the quantification of their risk

is challenging (Delfosse, 2005). This work represents the first record of populations of *G. holbrooki* in Argentina, *c.* 70 years after its introduction, suggesting that introductions have been done indiscriminately in this region, with no monitoring of results. Troubling newspaper reports from Argentina show that some health officials had recently released fishes to fight the transmission of dengue fever (New Report, 2012*a*, 2012*b*, 2016). Argentinian authorities should control and monitor this practice rigorously.

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Supporting Information

Supporting Information may be found in the online version of this paper:
Appendix S1. Examined material with a brief description of *G. holbrooki* collected in Argentina.

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