

Research Article

A newly identified population of *Gambusia affinis* (Baird and Girard, 1853), a non-native invasive species, in Lake Kenyir, Malaysia: implications for management

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Abstract

Gambusia affinis (Baird and Girard, 1853), a notorious non-native invasive fish species, has negatively impacted aquatic ecosystems around the world. This species was recently identified in Lake Kenyir, one of the largest impoundments in South East Asia, using DNA barcoding. The *cox1* sequence of *Gambusia* caught in Lake Kenyir was compared with the sequences of topotypic voucher specimens of *G. affinis* and two other candidate Poeciliidae. The species was found to cluster with *G. affinis* but not with monophyletic clades of either *G. holbrooki* or *P. reticulata* thus confirming species identity. The fish is yet to be widely established in the lake with the current distribution limited to areas of anthropogenic disturbance.

Key words: colonization, introduction, trophic overlap, competition, Dengue fever, mosquito, genetic barcode

Introduction

Gambusia affinis (Baird and Girard, 1853), commonly referred to as the mosquitofish, is native to the fresh waters of the southern United States and Mexico (Wooten and Lydeard 1990). *Gambusia affinis*, and a cryptic species *Gambusia holbrooki* Girard, 1859, were widely introduced at the beginning of the last century as a biological control agent for mosquitoes (Washino 1969; Sholdt et al. 1972; Green and Imber 1977) and mosquito-borne diseases (Russell 1993; Ghosh and Dash 2007). This practice remains widespread, however the effectiveness of *Gambusia* in the control of mosquito populations, despite its common name, has been questioned and it may be no more effective than native predators (Wilson 1960; Arthington and Lloyd 1989). *G. affinis* has

consequently become a pest in numerous waterways around the world and has been implicated in the declines of native fish populations through mechanisms which include competition for trophic resources, interference competition and predation (Pyke 2008). Due to the scale and severity of its impacts *G. affinis* was recently nominated as one of the world's worst 100 invasive species (IUCN ISSG 2013).

The documentation of the distribution of this fish is essential to enable managers to anticipate potential ecological issues that may arise after its introduction, mitigate the effects of its introduction and prevent its further spread. In the context of tropical systems, where biodiversity is generally high, knowledge of invasive species introductions may help to limit the loss of biodiversity i.e. species extinction.

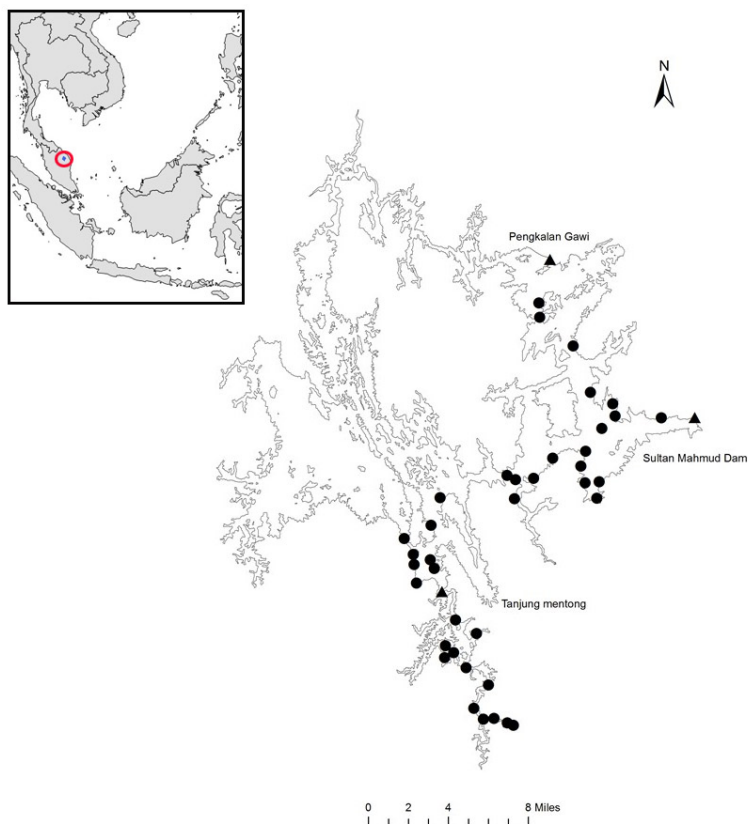


Figure 1. Location of Lake Kenyir within South East Asia (insert) and a map of the lake showing the distribution of sampling locations. Circles indicate an apparent absence of *G. affinis* and black triangles indicate observed presence of *G. affinis*, confirmed by sample collection and identification (for details see supplementary Table S1).

Although the presence of *G. affinis* is known in Malaysia (Lim and Tan 2002; Khairul et al. 2013), no official records of its introductions or distribution in the country have been published.

This study reports the first record of a newly identified population of *G. affinis* in South East Asia's largest man-made lake, documents genetic information from this population, and discusses the potential implications for lake management.

Materials and methods

Study site

Lake Kenyir was formed by the damming of the Terengganu River in 1986 to create the Sultan Mahmud Hydro Electric Power Plant and is the largest man-made lake in South East Asia covering 370km² (Furtado et al. 1977) (Figure 1). Lake Kenyir and its surrounding tropical forest is home to an incredibly diverse array of flora and fauna with an estimated 132 dipterocarp tree species, 290 species of birds and 61 recorded species of

fish. Some of these species are rare and endemic to this area. Lake Kenyir is a popular ecotourism destination, with thousands of people visiting each year to take part in activities such as sightseeing, fishing, swimming, jungle trekking and bird watching. In addition to tourism, the local economy is driven by commercial fishing and fish production (cage culture). Overfishing, unregulated stocking and aquaculture activities threaten endemic species through the depletion of fish numbers by capture, competition from introduced species (e.g. Tilapia, *Oreochromis mossambicus* (Peters, 1852) and Barramundi *Lates calcarifer* (Bloch, 1790)), disease and infection from cage culture and pollution. Neither *G. affinis* nor any other Poeciliidae have been reported in Lake Kenyir prior to this study despite extensive fish sampling taking place since the early 1990s, including methods that would detect small fish (e.g. electrofishing and hand nets) (Yap 1992; Department of Fisheries 1994; Yusoff et al. 1995; Zakaria et al. 1997; Ambak and Jalal 1998; Ahmad et al. 2002; Kamaruddin et al. 2011). Whilst common

fishing methods (e.g. gill nets), would not have been suitable to detect small fish such as *Gambusia*, these fish are generally conspicuous in clear-water marginal habitats due to their preference for the upper layers of the water column and it is therefore likely they would have been observed and documented during previous surveys if they were present.

Sampling

Visual observations of *Gambusia* were first performed during routine visits to Lake Kenyir on 9 December 2014 and 18 February 2015. These coincidental observations prompted timed (3 minute) visual observation surveys to be performed at 42 sites in the east and south areas of the lake (Figure 1 and Table S1) on 17–19 March 2015. Marginal habitats (<3 m from the bank) were targeted as *Gambusia* generally occupy shallow marginal areas within the littoral zone of lakes (Miura et al. 1979) especially where they are well-vegetated (Moyle and Nichols 1973) with low flow velocity (Pyke 2005). Efforts to catch individuals suspected of being *Gambusia* were made using a micromesh hand net. All captured individuals were measured (standard length, mm) and weighed (g). To verify the identity of Lake Kenyir specimens, fin clips of several samples were taken and preserved in 95% ethanol for subsequent DNA analysis. A representative specimen was caught, euthanized in a dilution of overdosed Tricaine Methanesulfonate and preserved in formalin for morphological description.

Identification and genetic analysis

G. affinis is often confused with other members of the Poeciliidae family, namely the eastern mosquito fish *G. holbrooki* and the guppy *Poecilia reticulata* Peters, 1859 which are also reported as introduced in Malaysia (Froese and Pauly 2015).

In order to identify Kenyir samples, the morphology of a voucher specimen was examined in the laboratory and the results compared with the descriptions of type material for the species (Rosen and Bailey 1963; Lloyd and Tomasov 1985). Morphometric measurements were taken using electronic calipers and were recorded together with meristic information. A general description of specimens is presented in this paper.

Morphological differentiation between *G. affinis* and *G. holbrooki* can be problematic and inaccurate due to individual variation in diagnostic features within species (Lloyd and Tomasov 1985;

Table 1. Length (SL) and weight of all individuals caught from Pengkalan Gawi on 9/12/2014 ($n = 5$), 18/2/2015 ($n = 3$) and 17/3/2015 ($n = 2$), Tanjung Mentong on 18/2/2015 ($n = 5$) and Sultan Mahmud Dam on 19/3/2015 ($n = 4$). Specimens are listed in chronological order of capture.

Site	Length (mm)	Weight (g)
Pengkalan Gawi	24	0.31
	21	0.14
	17	0.07
	18	0.08
	18	0.06
	18	0.06
	16	0.06
	16	0.03
	35	0.56
	22	0.08
	Tanjung Mentong	25
18		0.09
22		0.20
21		0.19
26		0.29
Sultan Mahmud dam	24	0.17
	22	0.12
	20	0.09
	20	0.08

Pyke 2005). Therefore a genetic analysis was performed to confirm species identification. Genomic DNA was extracted from a fin clip sample using Qiagen Tissue DNA Extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendation. Then, the 5' region of the cytochrome c oxidase subunit I (*cox1*) gene was amplified using universal primers (Wardet et al. 2005) and sequenced. The specimen *cox1* sequence was aligned with *cox1* sequences from topotypic voucher specimens of the three species (Table S2) using TranslatorX (Abascal et al. 2010). FastTree (Price et al. 2009) and Figtree v.1.4.2 (Rambaut 2009) were used for phylogenetic tree construction from the aligned sequences and tree visualization, respectively.

Results

Large shoals (>100 individuals) of *Gambusia* were first observed at Pengkalan Gawi on the East side of the Lake (5°08'16.7"N, 102°48'07.0"E) during a routine visit on 9 December 2014; five individuals were also caught using a hand net (Table 1). *Gambusia* were also observed at Pengkalan Gawi and Tanjung Mentong (4°54'2.0"N, 102°43'24.0"E) ($n = >50$ and >100 , respectively) and caught ($n = 3$ and 5 , respectively) during a subsequent routine visit to the lake on 18 February 2015 (Table 1). *G. affinis* were again observed



Figure 2. Image of *Gambusia affinis* from Lake Kenyir. Photograph by S.E. Walton.

($n = <20$) and caught ($n = 2$) at Pengkalan Gawi on 17 March 2015 and observed ($n = >20$) (not caught) at Tanjung Mentong on 19 March 2015. *G. affinis* were also observed ($n = <10$) and caught ($n = 4$) from one other site on this occasion; Sultan Mahmud power station dam wall ($5^{\circ}01'36.0''N$, $102^{\circ}54'25.0''E$) (Figure 1 and Table 1).

Identification

Morphological description

Upon examination, the back of all specimens was only slightly arched and the belly deep in front of the anal fin. The head was large in comparison to the body with a flattened upper surface, the mouth small upturned and protrusible and not reaching the front of the eyes. The eyes were very large relative to body size. The single, soft-rayed dorsal fin was short-based, high and rounded. The caudal fin was also rounded and the caudal peduncle long, deep and compressed. The head and trunk were covered in with large scales. In terms of colouration in life, the back was olive to brownish, the sides grey with a bluish sheen and the belly lighter. Females had a noticeable black patch above and somewhat forward of the vent. The eyes were greyish to olive. The dorsal fin (6–7 rays) and caudal fin had small black spots arranged into several indistinct cross rows in the latter. The anal fin (9–10 rays), pelvic fin (6 rays) and pectoral fin (11–12 rays) were of a translucent pale amber. Males were smaller than females and easily recognizable by the presence of a gonopodium. The specimens captured in Lake Kenyir differed from the description provided by McDowall (1990) who stated “there is no lateral line”, but a lateral line was clearly visible. There was a marked absence of coloured spots, however, the colouration of this species is known to be variable (Froese and Pauly 2015) (Figure 2). Counts of dorsal fin and anal fin rays can be used to differentiate *G. affinis* and *G. holbrooki*

(Lloyd and Tomasov 1985). However in this case variation in fin ray counts was such that the specimens could not be assigned to one particular taxonomic designation.

Genetic identification

Our sequenced *Gambusia* specimen displays 99–100% similarity to various *G.affinis cox1* sequences in GenBank database (accessed on 2nd April 2015). The taxonomic assignment of Kenyir specimens as *G. affinis* is further supported by phylogenetic clustering with *G.affinis* voucher specimens (Figure 3). The sequence has been deposited in Genbank and assigned the accession number KP756927.

Discussion

Through a combination of morphological examination and genetic analysis, the Poeciliidae captured in Lake Kenyir were confirmed to be *G. affinis*. Although morphological examination was inconclusive, the species was found to cluster with *G. affinis* but not with monophyletic clades of the either *G. holbrooki* or *P. reticulata* in a phylogenetic tree produced from *cox1* sequences, thus confirming species identity.

The mechanism of introduction into Lake Kenyir is not known, but it is speculated to be anthropogenic in nature and deliberate given the proximity of their detections to access points and human habitation and the reputation these fish have in combatting mosquitos (Hoy et al. 1972; Green and Imber 1977; Russell 1993) and mosquito-borne diseases (Russell 1993; Ghosh and Dash 2007). The occurrence of Dengue fever has increased dramatically in Malaysia in recent years (Loh and Sue-Chern 2014) and this introduction may have been an officially sanctioned or vigilante attempt to reduce the proliferation of the disease. Deliberate introductions into Lake Kenyir have occurred in the past, including *Oreochromis mossambicus* and *Lates calcarifer*, introduced to improve recreational angling but populations are not self-sustaining (Ahmad pers. comm.). Regardless of the mechanism or purpose of introduction, *G. affinis* is a notorious invasive species, and their impacts on other species around the world are well documented (Pyke 2008). This species poses a threat to Kenyir’s native species population viability through trophic competition, interference competition and by direct predation on other fish. This may have possible detrimental implications for socially and economically important commercial,

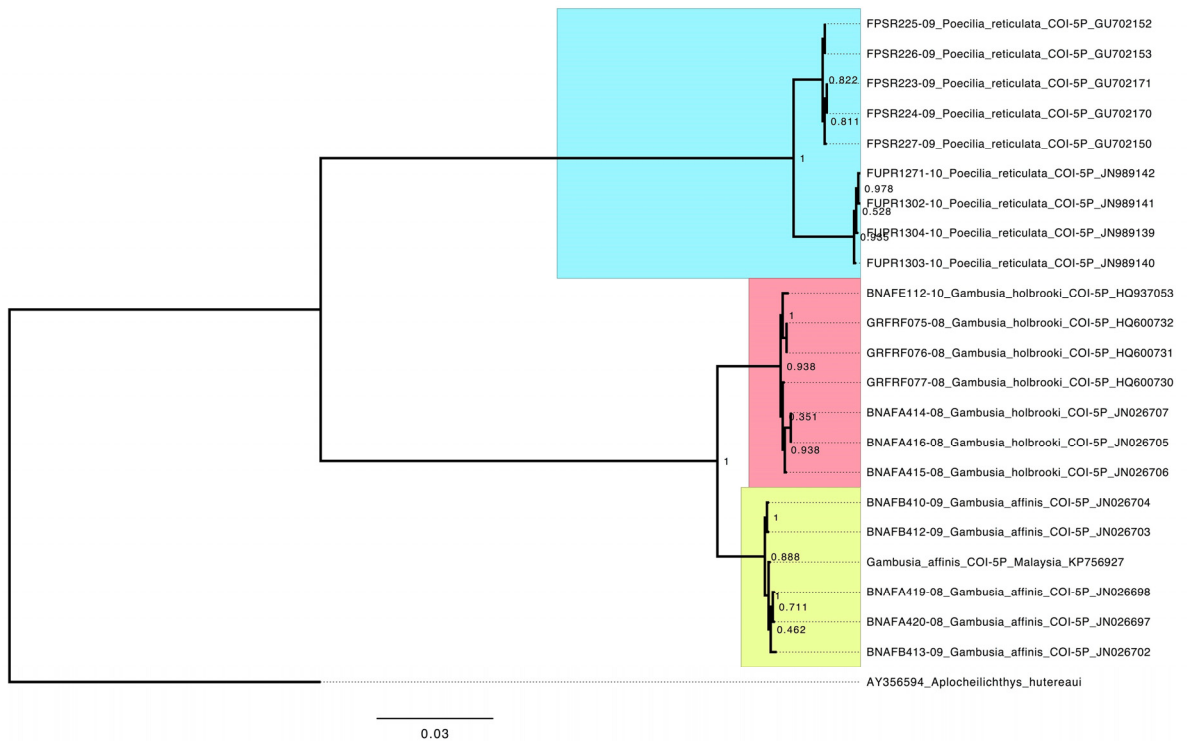


Figure 3. Phylogenetic tree depicting the evolutionary relationship of members from two *Gambusia* species and its close relative *Poecilia reticulata*, the common Guppy, based on *cox1* gene. Specimen numbers for the Barcode of Life Database (Ratnasingham and Herbert 2007) are shown next to each sample. The tree was rooted with *Aplocheilichthys hutereaui* as an outgroup.

subsistence and recreational fisheries the lake supports and management measures are considered.

Gambusia populations have been implicated in declines and disappearances of a number of fish species of similar size and habitat use (Arthington 1989; Courtenay and Meffe 1989; Galat and Robertson 1992). Therefore fish species in Lake Kenyir with similar niches as *G. affinis* are under particular threat due to interspecific competition for resources. Although the species-specific ecology of Kenyir's fishes is not well understood, several species are known to occupy similar preferred habitats to *G. affinis*. *Rasbora* sp. for example, occupy surface waters in low flow velocity areas, much like *G. affinis* (Pyke 2005) but the level of competition for trophic resources is not known. In the absence of detailed, locally relevant species-specific dietary information for Kenyir's fishes, the potential competition for food resources between sympatric species of the lake's littoral zone can only be estimated by similarity in trophic level estimates from a literature review (see Appendix 1). Whilst overlap in trophic levels between some species

could suggest competition for similar food resources (Appendix 1), without locally relevant data on the diet composition of *G. affinis* and co-occurring native species it is not possible to assess the true level of resource competition as the electivity of food items can differ within species of similar trophic level, affecting competition by resource partitioning.

More directly, *G. affinis* can severely impact native fish populations by preying upon eggs, juveniles and small individuals (Blaustein 1991; Schaefer et al. 1994; Pyke 2008). Direct predation on the eggs and young-of-year of economically important native species, such as Tinfoil barb *Barbonymus schwanenfeldii* (Bleeker, 1854) that spawn in the preferred habitat of *G. affinis*, may reduce recruitment, affecting commercial harvests in the future. A recent study has also shown that aggression by *Gambusia* can force other fish into habitats where there is increased exposure to other predators or decreased access to food (Ayala et al. 2007). Species designations for *Rasbora* sp., and many other fishes in Kenyir,

are yet to be conclusively determined. Therefore if interspecific interactions are found to be detrimental to endemic species, as in other reported cases (e.g. Meffe et al. 1983; Meffe 1985), species may be lost before they are described.

In contrast, Kenyir's predatory species may benefit from the introduction of *G. affinis*. The abundance of fast breeding and small *G. affinis* could present a source of food for piscivorous fishes in an artificial lake environment that may otherwise be underexploited by poorly adapted native species. Kenyir's most common predatory species, *Channa micropeltes* (Cuvier, 1831) and *Channa striata* (Bloch, 1793), are nest guards and clutch tenders (Phen et al. 2005). Thus juveniles may be less vulnerable to predation or aggression from *G. affinis*. The presence of predatory fish may, alongside food availability, limit the *Gambusia* population size (Hildebrand 1919). However the factors that regulate invasive *Gambusia* populations are complex and quantitative understanding of their population dynamics is poor (Pyke 2008).

The ecological impacts of the introduction of *G. affinis* will directly affect the ability of the ecosystem to provide the goods and services needed, or desired, by humans. Ecotourism is an important factor contributing to the socioeconomy in the region (Yusof et al. 2011). As the value of an ecotourism resource is derived from its perceived "naturalness", the presence of non-native invasive species and their potential impact on native biodiversity could therefore decrease this value (Higham 2007). The perception of recreational anglers may be particularly relevant in this case as they represent the largest demographic of lake users. Rumors of reduced quality of sport or experience can reduce angling participation with negative implications for the local economy (Veicht and Clout 2001). Conversely, the presence of invasive species can improve the output and reputation of a recreational fishery as growth rates of sought after fish are shown or perceived to be higher from predation on the invaders, resulting in desirable opportunities to catch bigger "trophy" fish (Veicht and Clout 2001).

Mosquitofish are difficult to eliminate once established (Hubbs and Brodrick 1963; Meffe 1985; Pyke 2008). The only documented cases of successful eradication of *Gambusia* from waterbodies were on a very small scale (e.g. surface area of <0.5km² (Mills et al. 2004)) and all attempts have also involved the total removal of native species using poisons (Pyke 2008). This kind of approach would not be feasible on a lake as large

as Kenyir. Given that the natural dispersal of this fish cannot be avoided in this case, the only way to reduce the impact of *Gambusia* in this region of Malaysia is to control their further spread through accidental or deliberate human translocation. A biosecurity control program to minimize accidental human translocation is recommended for the region, similar to the "check, clean, dry" initiatives employed in the United Kingdom, New Zealand and elsewhere (MPI 2013; GB NNESS 2014). The requirement for mosquito control in this area is not great but authorities and locals should nevertheless be educated on the possible implications of introducing these fish as part of integrated mosquito control programs (Ghosh and Dash 2007) to minimize deliberate human translocation. Further study of the interactions between native species and *G. affinis*, particularly with regards to resource competition, is recommended for the formulation of appropriate conservation measures.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Details of surveys conducted in March 2015 in Tasik Kenyir: location name and coordinates, habitat features, presence of *Gambusia affinis*, habitat features and water quality parameters.

Table S2. List of sequences used in genetic analysis from BOLD.

Appendix 1. Interspecific similarity in trophic level between *G. affinis* and sympatric species.

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